

Lincoln University Digital Dissertation

Copyright Statement

The digital copy of this dissertation is protected by the Copyright Act 1994 (New Zealand).

This dissertation may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- you will use the copy only for the purposes of research or private study
- you will recognise the author's right to be identified as the author of the dissertation and due acknowledgement will be made to the author where appropriate
- you will obtain the author's permission before publishing any material from the dissertation.

**Anesthesia methods and the agroecology of *Scaptomyza flava*
(Drosophilidae), a Brassicaceae pest in New Zealand and associated
parasitoid, *Asobara* nr. *persimilis* (Braconidae)**

A Dissertation
submitted in partial fulfilment
of the requirements for the Degree of
Doctor of Philosophy in Ecology

at
Lincoln University
by
Ryan James Rayl

Lincoln University
2018

Abstract of a Dissertation submitted in partial fulfilment of the
requirements for the Degree of Doctor of Philosophy in Ecology.

Agroecology of a salad crop pest in New Zealand

by

Ryan James Rayl

A key pest of brassicas worldwide is the leaf-mining fly, *Scaptomyza flava*, the larvae of which can cause cosmetic damage leading to crop rejection by supermarkets/consumers. In climates where leafy salad brassicas are harvested all year, the flies are almost always present. This necessitates control throughout the entire harvesting season and this usually consists of the prophylactic application of insecticides. One way of potentially ameliorating the negative environmental impacts of this approach is to enhance the effectiveness of biological control by providing alternative food sources for natural enemies. Planting of selected flowering plants can be useful in this respect, as many parasitoids and other beneficial insects feed on nectars. This in turn, can improve their efficacy by increasing fecundity, longevity and other aspects of their biology, contributing to increased 'fitness' and efficacy. This PhD aimed to find selective flowering plants that provided more benefit to the natural enemy when compared with the pest. This work was conducted through a series of laboratory and field experiments. Before the selection of flowering plants occurred, it was clear that the common practices facilitating handling drosophilid flies (carbon dioxide and chilling) were not appropriate for this work as it had been found that those methods can impact on the longevity of some insects. So, a series of experiments was conducted to compare alternative handling methodologies. Triethylamine (TEA) was compared with chilling and carbon dioxide because it had been shown in the literature to have some success but was still poorly studied and had not been used specifically to handle insects for ecological studies. Carbon dioxide and chilling were common in the literature as most studies that handled insects used one of these two. It was found in this work that triethylamine does not affect longevity and has a long anesthetic effect on the flies. So, TEA was used to handle the insects for the bioassays with flowering plants. Once this aspect of the work had been completed, female *S. flava* and one of its natural enemies, the braconid parasitoid wasp *Asobara sp. (nr. persimilis)* were used to evaluate six cultivars of alyssum (only the fly was used for these cultivars) and four flowering plant species (alyssum, buckwheat, phacelia and *Leptinella dioica*, Hook.f.). The latter is an endemic New Zealand plant in the Rosaceae. Cultivars differed from each

other in their effect on the fly and on *A. sp. (nr. persimilis)*. Buckwheat appeared to enhance longevity of the fly and the natural enemy to the greatest degree. The crop was *Brassica juncea* L. 'Mizuna'. Problems associated with the frequency distribution of the data meant that although Cox's proportional hazard model was initially used to compare survival times on different flowers, monotone likelihood made it difficult to ascribe statistical significance to the comparisons. Rankings, however, were consistent for the pest and *Asobara* and conclusions were drawn with appropriate caution. Because of the somewhat equivocal nature of the data and results, an analysis of variance was conducted to provide more robustness to the conclusions drawn. It seemed that none of the flower species evaluated was more beneficial to the parasitoid than to the pest. However, models have shown that as a parasitoid's attack rate is the most important parameter in that natural enemy's biocontrol potential, no differences between the effects of the 'best' flower (buckwheat (*Fagopyrum esculentum*) between the pest and the parasitoid) do not mean that other parameters which could have been recorded for that and other plants do not differ in their relative contributions to potential 'fitness' of the parasitoid and its host in the studied system. Attack rate *a sensu* Bailey et. al. has been shown to be the most important variable in this respect and can improve when buckwheat nectar is provided to some parasitoids. In the field, insect sampling targeting *S. flava* and its parasitoid occurred before and after buckwheat was sown to obtain information on a wide range of potential natural enemies of *Scaptomyza* and how they are affected by the buckwheat. A marking technology (rubidium chloride) was used to investigate patterns of potential natural enemy numbers in relation to that plant's flowers. Analysis was also carried out on the invertebrate natural enemy communities in that area. Overall, the work was the first time that these research questions had been applied to *S. flava* and useful ideas for future biological control work on this pest were demonstrated.

Keywords: Agroecology, anesthesia, *Asobara sp. (nr. persimilis)*, carbon dioxide, chilling, conservation biological control, Diptera, *Brassica juncea*, flowering plants, GIS, Hymenoptera, leaf miners, longevity, parasitoids, resource subsidies, *Scaptomyza flava*, survival analysis, triethylamine, tri-trophic interactions, trophic cascades,

Acknowledgements

I gratefully acknowledge funding support from Callaghan Innovation, the Bio-Protection Research Centre, Lincoln University, and Ashley Berrysmith of Snap Fresh Foods. Ashley generously supported this work throughout the study, despite financial challenges in his business. David Saville of Saville Statistical Consulting Ltd provided valuable advice on experimental design and Janine Johnson provided valuable support throughout the revision process. I would also like to thank my family and friends, especially my partner Júlia Pásztor for her help in various aspects during this period as well. Finally, I would like to thank my colleagues Sundar Tiwari, Dr. William Godsoe, and Morgan Shields for their various contributions throughout this thesis. Lastly, I would like to greatly thank my supervisor Prof. Steve Wratten for all his help with formulating experiments, ideas and all his help throughout the revision process.

Table of Contents

Abstract	i
Acknowledgements	iii
Table of Contents	iv
List of Tables	vii
List of Figures	ix
 Chapter 1 Introduction	 1
1.1 Overview	1
1.2 History of pesticide use.....	2
1.3 Whole-system approaches	2
1.4 Trophic cascades: enhancement through resource subsidies	3
1.5 Study system	4
 Chapter 2 Conservation biological control of insect pests: Benefits, limitations and implementation.....	 6
2.1 Introduction	6
2.2 The practicalities of conservation biological control	7
2.2.1 Non-consumptive effects in conservation biological control	8
2.2.2 The importance of long-term studies	10
2.3 Spatial scales from plots to agro-ecosystems.....	11
2.4 Interactions with the landscape	14
2.5 Conservation biological control in changing climates	15
2.6 Multiple ecosystem services.....	17
2.7 Ecosystem dis-services.....	19
2.8 Implementation of conservation biological control: From outputs to outcomes	20
2.9 The future of conservation biological control	22
 Chapter 3 A comparison of anesthesia techniques for entomological experimentation: Survival of the leaf-mining fly pest <i>Scaptomyza flava</i> Fallén (Diptera: Drosophilidae)	 24
3.1 Introduction	24
3.1.1 Carbon dioxide as an insect anesthetic.....	25
3.1.2 Chilling as an insect anesthetic	25
3.1.3 Triethylamine as an insect anesthetic.....	26
3.2 Materials and Methods.....	26
3.2.1 Range finders	27
3.2.2 Group 1 - Optimal treatments experiment.....	28
3.2.3 Group 2 - Final comparison experiment	30
3.3 Analysis	30
3.3.1 Group 1 analysis.....	30
3.3.2 Group 2 analysis.....	31
3.4 Results.....	31
3.4.1 Range finders	31
3.4.2 Group 1 - Optimal treatments	31
3.4.3 Group 2 - Final comparison experiment	32

3.5	Discussion.....	34
 Chapter 4 Inter- and intra-specific comparisons of plant nectar on the relative longevities of the fly pest <i>Scaptomyza flava</i> Fallén and its parasitoid, <i>Asobara</i> sp. (nr. <i>persimilis</i>) Papp.....		
4.1	Introduction	36
4.2	Materials and Methods.....	37
4.2.1	Alyssum cultivar evaluations with <i>Scaptomyza flava</i>	38
4.2.2	Flowering plant species evaluations with <i>S. flava</i>	39
4.2.3	Flowering plant species evaluations with <i>A. sp. (nr. persimilis)</i>	39
4.2.4	Analysis.....	40
4.3	Results.....	41
4.3.1	Alyssum cultivar evaluations with <i>Scaptomyza flava</i> (survival analysis)	41
4.3.2	Flowering plant species evaluations with <i>S. flava</i> (survival analysis)	42
4.3.3	Flowering plant species evaluations with <i>A. sp. (nr. persimilis)</i> (survival analysis) ...	43
4.3.4	Alyssum culitvar evaluations with <i>Scaptomyza flava</i> (ANOVA)	44
4.3.5	Flowering plant species evaluations with <i>S.flava</i> (ANOVA)	45
4.3.6	Flowering plant species evaluations with <i>Asobara sp. (nr. persimilis)</i> (ANOVA)	45
4.4	Discussion.....	45
 Chapter 5 Creating the big picture: Abundance, distribution and movement of a leaf mining pest fly, <i>Scaptomyza flava</i> Fallén (Diptera: Drosophilidae) and its potential natural enemies in salad crops.....		
5.1	Introduction	48
5.2	Materials and Methods.....	49
5.2.1	Field sampling	49
5.2.2	Measuring rubidium.....	50
5.2.3	Spatial abundance and distribution	51
5.2.4	Analysis.....	51
5.3	Results.....	51
5.3.1	Background concentrations of rubidium	51
5.3.2	Marked specimens.....	51
5.3.3	Distribution and abundance maps.....	51
5.4	Discussion.....	54
 Chapter 6 A survey of potential natural enemies of <i>Scaptomyza flava</i> obtained from yellow sticky traps related to distance from flowering buckwheat strip		
6.1	Introduction	56
6.2	Materials and methods.....	57
6.2.1	Keys	58
6.2.2	Analysis.....	58
6.3	Results.....	58
6.3.1	Sticky traps (small Hymenoptera)	58
6.3.2	Sticky traps (generalists)	58
6.3.3	Reared Hymenoptera (BHU)	59
6.3.4	Reared Hymenoptera (Auckland).....	59
6.4	Discussion.....	60
6.4.1	Sticky trap wasps.....	60
6.4.2	Generalist natural enemies on sticky traps.....	61
6.4.3	Material from BHU yellow traps	62
6.4.4	Material reared from Auckland.....	62

6.4.5	Final remarks.....	63
Chapter 7	General Discussion.....	80
7.1	Main findings and their implications	80
7.2	Conclusions	82
Appendix A	Supplementary tables and figures.....	84
References	87

List of Tables

Table 3.1 Power analysis for the experiments that observed recovery time between rates of each anesthesia technique (Group 1). Threshold recovery time (15 min) was the variable that determined the number of replicates required.....	27
Table 3.2 Results from comparing the three anesthesia techniques (Group 2 experiment) showing the estimates for the median recovery and median deaths with their respective lower and upper confidence limits. The anesthesia treatment chosen is highlighted in bold. Two treatments are selected because anesthesia type, not effect was the category of interest. The highest median recovery time was used to determine the best treatment because there was no significant difference between the longevities across all treatments.	34
Table 4.1 A comparison between alyssum cultivars for <i>Scaptomyza flava</i> longevity. The upper confidence limits have not been reported because of monotone likelihood (see Section 4.2.4). The cultivar with the highest median survival time (hereafter the best) is denoted by an asterix (*).	41
Table 4.2 Longevity of <i>Scaptomyza flava</i> on five species of flowering plants. The upper confidence limits have not been reported because of monotone likelihood (see section 4.2.4). The species resulting in the highest median survival time (hereafter the 'best') is denoted with an asterix (*).	42
Table 4.3 <i>Asobara</i> sp. (nr. persimilis) longevity on different flowering plant species. The upper confidence limits have not been reported because of monotone likelihood (see Section 4.2.4). the criterion to designate the best species was the same as Tables 1 and 2. The species with the highest median survival time is denoted with an asterix (*).	43
Table 4.4 ANOVA table results of survival means for <i>Scaptomyza flava</i> on six different <i>L. maritima</i> cultivars and water controls. Data was non-parametric so a log transformation was used and then data in the second mean column was back-transformed for relatability.....	44
Table 4.5 ANOVA table results of survival means for <i>Scaptomyza flava</i> on four different plant species and water controls. Data was non-parametric so a log transformation was used and then data in the second mean column was back-transformed for relatability.....	45
Table 4.6 ANOVA table results of survival means for <i>Asobara</i> sp. (nr. persimilis) on three different plant species and water controls. Data was non-parametric so a log transformation was used and then data in the second mean column was back-transformed for relatability.....	45
Table 6.1 Numbers of Hymenoptera captured on yellow sticky traps at exponential distances from a flowering strip of buckwheat (<i>Fagopyrum esculentum</i>). For position zero, traps were within the flowering buckwheat. The work was done at the Biological Husbandry Unit, Lincoln University, Lincoln, New Zealand and the traps were collected April 16 th 2016.....	59
Table 6.2 Counts of insect generalist natural enemies found on yellow sticky traps at exponential distances from <i>Fagopyrum esculentum</i> . The work was done at the Biological Husbandry Unit, Lincoln University, Lincoln, New Zealand and the traps were collected April 16 th 2016.....	60
Table 6.3 Individual hymenopteran counts per family reared from <i>Brassica juncea</i> 'Mizuna' leaves taken from crop plants at the Biological Husbandry Unit, Lincoln University, Lincoln, New Zealand. See Materials and methods section and the traps were collected April 16 th 2016.	60
Table A.1 Preliminary experiments to find a range of variables in which to test each anesthesia method.	84

Table A.2 Results from the Group 1 experiment. The estimates for the median recovery with the lower confidence limits and the mean deaths per replicate are given. The upper confidence limits are not reported because they were unbounded because of monotone likelihood. The anesthesia treatments chosen for Group 2 are highlighted in grey. The highest median recovery time and lowest death rate were used to determine the best treatments.85

List of Figures

Figure 3.1 Foam-plugged 35ml vial with straw and swab inserted for application of triethylamine (A), meshed cap (B) Eppendorf tube filled with water and with a cotton-wool plug. A similar vial setup but no swab and straw was used when applying CO ₂ . The vial with a foam plug (A) was floated in a water bath for the chilling treatments. All treatments used the vial depicted in C for data collection.	29
Figure 3.2 Kaplan-Meier analyses of time to recover from anesthesia techniques. The curves for the sexes are similar for CO ₂ and chilling but different in triethylamine (TEA). The graph for controls is not shown as it would be superimposed on the y-axis.....	33
Figure 3.3 Survival rates of <i>Scaptomyza</i> in the three anesthetic treatments and the control. TEA = trimethylamine.	33
Figure 4.1 A chamber used to isolate a female <i>Scaptomyza flava</i> with an inflorescence. All containers had an Eppendorf tube filled with water and plugged with cotton for insect hydration.	39
Figure 4.2 Kaplan-Meier survival curves of <i>Scaptomyza flava</i> adults on six flowering <i>Lobularia maritima</i> (alyssum) cultivars (see Table 1). The control line (black) reaches the x-axis at 4 days, as does that for ‘Tiny Tim’ (yellow line).	42
Figure 4.3 Kaplan-Meier survival curves of <i>Asobara</i> sp. (nr. <i>persimilis</i>) adults on 3 different flowering plant species (see Table 4.3).	44
Figure 5.1 The maps display a ‘snapshot’ of the fly mines per leaf in late December 2015 (left) before the buckwheat was sown and the fly mines per leaf 6 weeks after buckwheat was sown, causing the sampling to occur in early April 2016 (right). The overall densities of the mines decrease with many of the “hotspots” in December completely evening out, while the highest densities in April are relatively close to the buckwheat.	53
Figure 5.2 The maps display a ‘snapshot’ of the wasps reared out of leaf material in late December 2015 (left) before the buckwheat was sown and the wasps reared out of leaf material 6 weeks after buckwheat was sown, causing the sampling to occur in early April 2016 (right). There were generally fewer wasps reared in December while in April there was a “hotspot” near the highest densities of the flies, which happens to be close to the buckwheat.	54
Figure 6.1 A female <i>Asobara</i> sp. (nr. <i>persimilis</i>) adult taken from established laboratory colonies.	64
Figure 6.2 An encyrtid (sex unknown) caught on the yellow sticky traps at the BHU.	65
Figure 6.3 A figitid (sex unknown) found on yellow sticky traps at the BHU. All figitid specimens found on sticky traps were <i>Anacharis zealandica</i> , including this specimen.....	66
Figure 6.4 A large ichneumonid (sex unknown) found on sticky traps at the BHU.	67
Figure 6.5 A <i>Diadegma semiclausum</i> (sex unknown) specimen found on yellow sticky traps at the BHU.	68
Figure 6.6 A megaspilid (sex unknown) found on yellow sticky traps at the BHU.....	69
Figure 6.7 A pteromaild (sex unknown) found on yellow sticky traps taken from the BHU.	70
Figure 6.8 The single torymid (sex unknown) found on the yellow sticky traps taken from the BHU.	71
Figure 6.9 A Brown lacewing (<i>Micromus tasmaniae</i>) found on yellow sticky traps taken from the BHU.	72
Figure 6.10 A <i>Vespula germanica</i> found on yellow sticky traps erected on the BHU. A – Face of <i>V. germanica</i> straight black line down centre of face is a distinguishing feature. B – Side view of <i>V. germanica</i> solid colour band behind eyes is a second identifying feature..	72
Figure 6.11 A <i>Vespula vulgaris</i> found on yellow sticky traps erected on the BHU. A – Face of <i>V. vulgaris</i> anchor shaped black mark down centre of face is a distinguishing feature. B – Side view of <i>V. vulgaris</i> band behind eyes interrupted by a black mark is a second identifying feature.	73

Figure 6.12 An eleven-spotted ladybird beetle (<i>Coccinella undecimpunctata</i>) found on yellow sticky traps taken from the BHU.....	73
Figure 6.13 An <i>Aphidius</i> sp. (Braconidae) reared from plant material harvest from Brassica juncea 'Mizuna' grown on the BHU plot.	74
Figure 6.14 A <i>Trichopria</i> sp. (Diapriidae) reared from Brassica juncea 'Mizuna' leaves harvest from plants grown on the BHU plot.	75
Figure 6.15 A <i>Spilomicrus</i> sp. (Diapriidae) reared from Brassica juncea 'Mizuna' leaves harvest from plants grown on the BHU plot.	76
Figure 6.16 A representative of all eulophids reared from plant material harvested from Brassica juncea 'Mizuna' grown on the BHU plot.....	77
Figure 6.17 A reared figitid from Brassica juncea 'Mizuna' material harvest on the BHU.....	78
Figure 6.18 A reared torymid from Brassica juncea 'Mizuna' material harvest from the BHU.	79
Figure A.1 Three-week-old Brassica juncea 'Mizuna' in a 'Bugdorm.' This is the stage at which <i>Scaptomyza flava</i> were introduced to the plants.....	84
Figure A.2 35 ml vials containing recovering <i>Scaptomyza flava</i> . 25mm diameter by 75 mm height.....	84
Figure A.3 Evidence that anesthesia information is sought after. This is a preprint and not a peer-reviewed article. This is important because even with this informal format, there is a high interest in it, lending weight that the publication of this material is of interest.	87

Chapter 1

Introduction

1.1 Overview

Current practices of prophylactic pesticide use in agroecosystems are detrimental to human health and the environment (Unruh *et al.*, 2012; Schmitz & Barton, 2014). Also, pests, weeds and plant pathogens quickly develop resistance that severely impacts on their effectiveness (Guedes, 2017). Alternative approaches to these high-input practices are being developed. One such research area manipulates the agroecosystem to favour pests' natural enemies (NE), so that pest suppression can be achieved with minimal inputs (Gurr *et al.*, 2017). The key components of this idea can be summarised by the acronym SNAP (Shelter, Nectar, Alternative food and Pollen; Gurr *et al.*, 2017). This approach is called conservation biological control (Cons BC) (DeBach, 1964; Barbosa & Wratten, 1996; Eilenberg *et al.*, 2001). Cons BC also closely ties into ecosystem services (ES), ecosystem functions from which humans derive value (Jax *et al.*, 2013). This is because many aspects of SNAP as well as enhanced biological control are directly valued by humans and this additional value makes Cons BC even more robust and attractive for implementation. Examples include aesthetics, pollination and soil improvement. Some well-established Cons BC techniques that have been vigorously examined within the scientific community include but are not limited to providing: refugia using 'beetle banks' (Thomas *et al.*, 1991) or other ecological enhancement schemes (EES) (Batory *et al.*, 2011), alternative food through food sprays, alternative prey/hosts or nectar/pollen and using trap crops (Khan *et al.*, 2011) creating barriers to pest movement (Messelink *et al.*, 2014; Roubos *et al.*, 2014; Gurr *et al.*, 2015; Sandhu *et al.*, 2016). Ideally, providing all the four components of SNAP would have an additive/synergistic effect that would greatly favour natural enemies over the pests and pest suppression would occur. This can also be referred to as enhancing trophic cascades using resource subsidies. Each pest/crop is unique and needs specific research to find the most appropriate combination of SNAP. One such technique is to sow flowering plants within and around agroecosystems (Lu *et al.*, 2014). Not only can these plants provide shelter, nectar and pollen for NE, but they can also provide refugia for alternative hosts/prey of the NEs (Lu *et al.*, 2014). Buckwheat is an example of a flowering plant that can also provide more than one ES that includes, but is not limited to: pest control, pollination, soil improvement and eco-tourism (Shields *et al.*, 2016). Conservation biological control, including its potential negatives, is explored in more detail in Chapter 2. The rest of this Introduction discusses in more detail a whole system approach to agriculture and then outlines the details of the study system used in this thesis and the approaches used.

1.2 History of pesticide use

The convention of widespread pesticide use was established for controlling pests with little cost and effort (Hillebrandt, 1958). However, pesticide use is often not economic over time because of environmental damage, non-target toxicity and the evolution of pest resistance, especially in invertebrates (Wardlow *et al.*, 1976; Ridgway *et al.*, 1978; Pimentel *et al.*, 1992; Hagenbucher *et al.*, 2016; Grigoraki *et al.*, 2017). The frequent prophylactic use of pesticides leads to pesticide resistance, increasing cost and effort for control (Mallet, 1989; Leibee & Capinera, 1995; Guo *et al.*, 1998; Wilson & Tisdell, 2001; Weston *et al.*, 2004, 2005; Abhilash & Singh, 2009; Hagenbucher *et al.*, 2016; Grigoraki *et al.*, 2017). Not only can the efficacy of pesticides decrease over time, but pesticide use can alter interactions in food webs and severely damage ecosystem functions (Weston *et al.*, 2004, 2005).

Many pesticide residues can move within and between ecosystems and persist in the environment through adsorption, bioaccumulation and leaching etc. (Pimentel *et al.*, 1992; Wilson & Tisdell, 2001; Weston *et al.*, 2004, 2005; Abhilash & Singh, 2009). Bioaccumulation is the process through which toxins in an environment accumulate as they move up trophic levels in food webs. Prey organisms can take up small concentrations of toxins that have no negative effects at a lower trophic level but which may be concentrated in predators, leading to losses of 'fitness' or death. Even small-dose exposure to pesticide residues can cause behavioural changes (Goulson, 2013), disease, illness or death in many species of organisms if exposed over long periods of time (Ridgway *et al.*, 1978; Wilson & Tisdell, 2001; Konradsen *et al.*, 2003; Abhilash & Singh, 2009; Araujo *et al.*, 2017). The cost of developing new pesticide molecules is also increasing drastically, partly because of the increasing time it takes to fulfil all the requirements to certify a pesticide (Carvalho, 2006; Abhilash & Singh, 2009). These challenges to pesticide use and their consequences are leading to increasing awareness and suggests that an alternative whole-system approach to pest management is needed.

1.3 Whole-system approaches

Integrated pest management (IPM) is a whole-system approach that has been tested and successfully implemented in many cropping systems, with the aim to reduce the environmental impacts of farming and to create an avenue for more sustainable agricultural practices (Bottrell & Smith, 1982; Blommers, 1994; Kogan, 1998; Demoranville *et al.*, 2000; Cook *et al.*, 2007; Birch & Begg, 2010). IPM focuses on the use of appropriate pesticides with limited effects on pests' natural enemies, crop scouting in relation to the economic injury level (EIL), host-plant resistance, transport in waterways and across the landscape etc. (Bottrell & Smith, 1982; Blommers, 1994; Kogan, 1998; Demoranville *et al.*, 2000; Cook *et al.*, 2007; Birch & Begg, 2010).

A successful IPM programme can reduce pest incidence, environmental impact and even pesticide use (increased accuracy and timing leads to less spraying overall) while increasing crop yield (Bottrell & Smith, 1982; Blommers, 1994; Kogan, 1998; Demoranville *et al.*, 2000; Cook *et al.*, 2007; Birch & Begg, 2010). It rarely includes habitat manipulation to enhance biocontrol effectiveness including conservation biological control (Cons BC) as this is a relatively new part of biocontrol science (Gurr *et al.*, 2017). In recent years, public and political opinion in Europe, Australia and the United States has tended to demand little to no pesticide residues on consumed products. This created a need to reduce pesticide use even further for export to these countries. Recently, Cons BC has become a popular concept as a means of enhancing biocontrol effectiveness as a key ecosystem service (ES) in farmland. ES are generally fundamentally important to ecosystem health and have a considerable value in that area (Costanza *et al.*, 2006; Wratten *et al.*, 2013). Cons BC focuses on restoring, enhancing, and sustaining biocontrol and possibly multiple ES, in response to the demand for few pesticide residues (De Schutter, 2009a, 2009b; Wratten *et al.*, 2012). Cons BC can work in association with some pesticides, but manages natural enemies which already exist in or around the target agroecosystem (SNAP).

1.4 Trophic cascades: enhancement through resource subsidies

In agroecosystems, predicting and suppressing pest outbreaks can be difficult because many variables are involved for successful control. Fortunately, when natural enemy populations are high, pest outbreaks become infrequent and pest damage to crops may fall below economic thresholds (ET) and EILs. Augmentation of natural enemy populations is an important aspect of Cons BC (Kean *et al.*, 2008). Resource subsidies and trophic cascades are relatively novel concepts that have been developed to augment natural enemy populations and/or efficacy (Tylianakis *et al.*, 2004; Winkler *et al.*, 2006; Araj *et al.*, 2008; Balzan & Moonen, 2014; Roubos *et al.*, 2014).

Trophic cascades are 'downstream effects' from higher trophic levels influencing at least two levels below it (Tschardt & Hawkins, 2002). For example, predation of herbivorous pests which leads to more plant biomass is a trophic cascade from level 3 to level 1 (Pace *et al.*, 1999; Schmitz *et al.*, 2000; Halaj & Wise, 2001). Trophic cascades can be enhanced through resource subsidies, which are energy or nutrient flows from a source which is normally outside the trophic system under study (Richardson *et al.*, 2010). A resource subsidy can increase the fitness of a beneficial organism and can even trigger a stronger cascade (Pace *et al.*, 1999). In the context of Cons BC, resource subsidies are included in SNAP (Gurr *et al.*, 2017).

1.5 Study system

Globally, many brassica species are grown for their leaves and are an important part of human diets; salad brassicas and leafy bok choy and its relatives are especially common in south and east Asia. These leaves are often processed to be packaged for supermarket fresh sales, which in New Zealand generated 53.7m NZD (~37.8m USD) in 2015/2016 (Wilkinson *et al.*, 2015). The major pest of these brassicas in NZ is a leaf mining fly, *Scaptomyza flava* Fallén in the family Drosophilidae. *S. flava* populations are suppressed mainly by using prophylactic pesticide applications. This control method dominates because the fly lays eggs on the surface of the leaf, the larvae emerge from the eggs and then burrow down through the leaf's surface and then start consuming the mesophyll cells, leaving white to brown mines. The early stages of this damage are not easily discernible with the naked eye (Seraj, 1994) and are considered cosmetic because they do not cause direct yield loss. These early serpentine mines are succeeded by blotch mines, as the larvae grow. However, such leaves are completely rejected in the pack house. This causes more pressure on the grower to generate unblemished leaves, which leads to more reliance on prophylactic pesticide application, even if the damage has not reached the threshold at which they cause noticeable/monetary damage. However, such thresholds for this pest are either so low as to be inoperable or have not been established.

This PhD project aimed to address this prophylaxis issue by attempting to develop a conservation biological control programme. Although there are no established ETs for this system, a Cons BC programme is still relevant because it can reduce the amount of damage done by the pests, which reduces the number/proportion of damaged leaves/plants. If successfully implemented, this reduces processing time and in turn decreases inputs and costs to the farmer. The focus of the research was finding candidate flowering plants (as mentioned above) to provide SNAP to NEs. In this specific agroecosystem, it was found that the main NE of *S. flava* was a parasitoid wasp, *Asobara sp. (nr. persimilis)* Papp, 1977 (Braconidae) (Martin, 2012). *A. sp. (nr. persimilis)* was chosen as the study NE because it had been mass reared and then released across New Zealand in 2009 (MacDonald *et al.*, 2011). *A. sp. (nr. persimilis)* is a larval and pupal endo-parasitoid that originated from Australia but has been found all over New Zealand's North Island throughout the year by being reared from *S. flava* pupae (Martin, 2004, 2012). *A. sp. (nr. persimilis)* parasitizes the larvae or pupae of *S. flava* and then kills the pupal stage before it can fully develop, this type of parasitoid is a koinobiont.

As a first step, identifications were conducted on *S. flava* individuals from the field to ensure that the biocontrol target was indeed *S. flava*. Morphological as well as molecular identification (DNA barcoding by amplifying the COI region of *S. flava*, Folmer *et al.*, 1994) both confirmed the specimens as *S. flava*. *A. sp. (nr. persimilis)* specimens were provided by Emma Barraclough at Plant and Food Research, Tamaki Campus, Auckland, NZ. *A. (nr. persimilis)* was then reared at Lincoln University.

Once the identifications were completed this work had two main components: laboratory and field experiments. The former were used to rear colonies of flies, refine insect anesthesia/handling methodologies, and find candidate flowering plant species for future field deployment. In the field, the abundance and distribution of the insects before and after buckwheat (*Fagopyrum esculentum* 'Katowase' Moench.) was deployed. The insects were also tracked using a mark and recapture methodology to try to determine their dispersal patterns after feeding on rubidium-labelled buckwheat nectar (Corbett *et al.*, 1996; Scarratt *et al.*, 2008). This PhD began as a new partnership with a New Zealand company (SNAP Fresh Foods) to innovate their agroecosystem management through a Callaghan Innovation Fellowship. The work conducted here was within the framework of conservation biological control. This type of approach has many challenges, as do the other two (classical and augmentative; Gurr & Wratten, 2000) and these and prospects for enhancing the efficacy of this method are reviewed in more detail in Chapter 2. In particular, biological control of a pest which spends a significant part of its life cycle make the work in this study a particular challenge.

Chapter 2

Conservation biological control of insect pests: Benefits, limitations and implementation

2.1 Introduction

Projections for the human population indicate that it will reach 9 billion globally by 2050 (Godfray *et al.*, 2012; Tscharntke *et al.*, 2012; Ingram *et al.*, 2013). Pesticides, fertilizers and irrigation have been key components of this growth to date (Cooper & Dobson, 2007; Tilman *et al.*, 2011), as in the ‘Green Revolution’ (Tilman, 1998). Prophylaxis has been a common approach (Unruh *et al.*, 2012; Schmitz & Barton, 2014) and reports to the United Nations (de Schutter, 2010; United Nations, 2017) confirm that this use of pesticide has caused external costs such as reduced human health and environmental issues (Sandhu *et al.*, 2012; Tscharntke *et al.*, 2012). This has led to a decrease in support for the use of pesticides, especially in the European Union (United Nations, 2017).

The demand for low-input alternatives to pesticides has arisen from issues with pesticide resistance, increasing costs and regulation, and emerging consumer pressure (Jetter & Paine, 2004; Grygorczyk *et al.*, 2014; Wollaeger *et al.*, 2015; Gurr *et al.*, 2017; United Nations, 2017). This has triggered a growing interest for policymakers to create programs that support sustainable growth (Fabinyi *et al.*, 2016; Gartaula *et al.*, 2016), sometimes called ‘sustainable intensification’ (Godfray *et al.*, 2010). One of these alternatives is manipulating the agro-ecosystem to make it more favorable for natural enemies, this is known as conservation biological control (CBC) (Begg *et al.*, 2017; Gurr *et al.*, 2017). Many techniques can be used under the umbrella of CBC to manipulate the agro-ecosystem in this way; some of these include floral resource augmentation (Figure 1) (Pywell *et al.*, 2015; Tschumi *et al.*, 2015; Gurr *et al.*, 2016), ‘beetle banks’ (a type of banker plant) (Thomas *et al.*, 1991; MacLeod *et al.*, 2004) or artificial food sprays (Seagraves *et al.*, 2011; Tena *et al.*, 2015). A key and well-used acronym which encompasses most aspects of how the environment can be manipulated under CBC is

SNAP: Shelter, Nectar, Alternative food and Pollen (Figure 1) (Gurr *et al.*, 2017). In recent work, the nectar and pollen components can be summarized by another acronym: BAP. This captures buckwheat, (sweet) alyssum and phacelia, which are often the most effective floral additions to crops, founded by prior laboratory bioassays. Although CBC is popular with the scientific community and many studies have explored different aspects of it, many unanswered questions remain. For example, to what extent do added resources act synergistically to provide multiple ES delivery or do they include elements of redundancy and competition, generating ecosystem dis-services?



Figure 1. *Lobularia maritima* 'Benthamii White' (alyssum) amongst spinach and lettuce crops in Auckland, New Zealand. The alyssum was planted to provide SNAP (Shelter, Nectar, Alternative food and Pollen) for parasitoid wasps that parasitize larvae of a leaf mining fly pest. Photo: Ryan Rayl.

2.2 The practicalities of conservation biological control

When applying CBC in an agro-ecosystem, the natural enemy-pest community needs to be assessed and manipulated in a range of ways. The acronym, ARMED, can be helpful in this process: Assess,

Rank, Manipulate, Evaluate, Deploy (Shields *et al.*, in press). A range of sampling methods exist to Assess which species comprise the invertebrate community in a particular crop and, to some extent at least, to which guilds the different taxa belong. The taxa can then be *Ranked* in a number of ways. The simplest being abundance. Populations and efficacy of some individuals or guilds can then be *Manipulated*, the effects of which then need to be *Evaluated*. After evaluation, the protocols developed to manipulate the arthropods need to be *Deployed* appropriately in agro-environment schemes, with effective delivery systems and pathways to implementation. In that context, Wratten *et al.* (in press) showed that although the number of agro-ecological publications has increased exponentially in recent decades, this has not usually led to many on-farm outcomes, with the exception of work by Khan *et al.* (2011) and Gurr *et al.* (2016). It is important to note that attempts at the ARMED process may reveal ecosystem dis-services (EDS) which at least partially negate the benefits (Tscharntke *et al.*, 2005; Bianchi *et al.*, 2006; Tschumi *et al.*, 2015; Rusch *et al.*, 2016; Gurr *et al.*, 2017). EDS in the context of CBC are specific unintended negative impacts from the added biodiversity and its processes (Zang *et al.*, 2007; Gurr *et al.*, 2017), such as added plants becoming weeds (Shields *et al.*, 2016).

Although this focus on floral resources has generated many successful manipulations that have reduced pest numbers (Géneau *et al.*, 2012; Tschumi *et al.*, 2015), much less work has been done in terms of alternative hosts or prey living on the provided flowering plants (Messelink *et al.*, 2014; Gurr *et al.*, 2015; Gillespie *et al.*, 2016). This aspect is represented by the A in SNAP. Furthermore, if some insect species feed on both the deployed plants and the adjacent crop, then targeting that potential EDS needs to be quantified.

2.2.1 Non-consumptive effects in conservation biological control

Non-consumptive effects (NCE) are changes in prey behaviour and physiology that improve their predator avoidance (Buchanan *et al.*, 2017; Hermann & Landis, 2017), which can impact pest management and influence entire agro-ecosystems through trophic cascades (Hermann & Landis, 2017). This emerging field of study has already found strong evidence that NCE may have a

substantial role in reducing pest damage and needs to be considered when developing protocols for CBC. Many studies have shown that prey respond to predators by changing either their behaviour or physiology to reduce the risk of predation. These include increased predator avoidance (Wratten, 1976; Hoefler *et al.*, 2012; Lee *et al.*, 2014), reduced feeding (Rypstra & Buddle, 2013; Kaplan *et al.*, 2014; Thaler *et al.*, 2014), reduced oviposition (Wasserberg *et al.*, 2013; Sendoya *et al.*, 2009) and changes in host plant preference (Wilson & Leather, 2012; Sidhu & Wilson Rankin, 2016).

To manipulate NCE in CBC, the specific mechanisms of predator detection must be understood. These are predominantly chemical cues (Gonthier, 2012; Hoefler *et al.*, 2012; Gonzalez & Rodriguez-Girones, 2013; Hermann & Thaler, 2014) such as aphids (*Rhopalosiphum padi* L.) having reduced colonisation on plants where ladybird beetle (*Coccinella septempunctata* L.) larvae had previously foraged (Ninkovic *et al.*, 2013). Another example is reduced feeding of Colorado beetles (*Leptinotarsa decemlineata* Say) when exposed to the predatory stink bug, *Podisus maculiventris* Say (Hermann & Thaler, 2014). However, visual detection of predators, while often undervalued, may contribute substantially to predator avoidance (Sendoya *et al.*, 1996). For instance, pollinator visitation decreased when models that look like crab spiders were on the flowers (Antiqueira & Romero 2016). Also, dried ants pinned to plants reduced butterfly oviposition (Sendoya *et al.*, 2009). One of the most important NCE that should be investigated in CBC manipulations is pests modifying their choice of host plant from the preferred hosts to one of lower nutrition, based on their perceived predation risk (Hermann & Landis 2017). For instance, in a mesocosm experiment, the grasshopper, *Melanoplus femurrubrum* De Geer, switched from preferred grasses to less nutritional forbs when the spider predator, *Pisaurina mira* Walckenaer, was present (Schmitz, 1998). The potential for manipulating NCE in CBC habitat manipulation and IPM protocols is enormous, such as in push-pull systems (Hermann & Landis 2017). However, very few studies have been conducted in the field at time scales longer than 1 week or investigated the impacts of NCE on agro-ecosystem functions and across different spatial scales (Hermann & Landis 2017).

2.2.2 The importance of long-term studies

Communities and landscapes change over time and with those changes comes the shifting of the natural enemy-pest community. Most studies to date have examined short-term effects (<3 years) in these systems. Gillespie *et al.*, (2016) and Gurr *et al.*, (2017) have both stressed the need for more long-term studies. Both agree that short-term studies cannot accurately capture population trends of the organisms in and around the agro-ecosystem. Providing SNAP in the form of annual flowering plants for one season only is the most common practice (Figure 1). Naturally-occurring SNAP, for example through perennial shelter, can operate more long term. Other examples are manipulated hedgerows (Holland *et al.*, 2016), beetle banks (Thomas *et al.*, 1991; MacLeod *et al.*, 2004) and long-term plantings of some biofuel crops (Figure 2) (Porter *et al.*, 2009; Littlejohn *et al.*, 2015). Short-term rotational coppice (Langer 2001; Rusch *et al.*, 2014) and plantings of the giant hybrid grass *Miscanthus x giganteus* (Greef et Deu) can provide refugia for natural enemies (Figure 2) (Semere & Slater, 2007; Shields *et al.*, in press) as well as providing other non-biological control refugia (Littlejohn *et al.*, 2015). However, there are many ecological mechanisms which impede the delivery of biological control from refugia and these are reviewed in Tscharntke *et al.* (2016). A good example is that in Europe, carabid beetles and other predatory fauna disperse into the adjacent crop from hedges and beetle banks in the spring (Thomas *et al.*, 1991; Holland *et al.*, 2016), while in New Zealand, those habitats provide refugia all year round with little emigration from them (McLachlan & Wratten, 2003).



Figure 2. *Miscanthus x giganteus* (Greef et Deu) shelterbelt on a dairy farm providing refugia for natural enemies, Canterbury, New Zealand. Photo: Chris Littlejohn.

2.3 Spatial scales from plots to agro-ecosystems

Research strongly suggests that monocultural agro-ecosystems are detrimental as they can lead to higher pest populations (Kremen & Miles, 2012; Nilsson *et al.*, 2016; Rusch *et al.*, 2016). Although much research supports agro-ecosystem diversification, recent evidence suggests that this can be a complex problem to address and is not as straightforward as increasing diversity in these agro-ecosystems. There is now considerable evidence suggesting that agro-ecosystem management should take place at the landscape scale because it can provide better arthropod management (Tscharntke *et al.*, 2007; Karp *et al.*, 2012; Chaplin-Kramer *et al.*, 2013; Roubos *et al.*, 2014). This is because it was found that at a landscape scale of at least 2.5 km, there was a strong relationship between natural habitat and the abundance of predatory flies (Chaplin-Kramer *et al.*, 2013).

While it is true that diverse cropping systems generally do lead to more natural enemies, fewer pests and less crop damage, in some cases yield can significantly decline (Jonsson *et al.*, 2010; Tscharntke *et al.*, 2016; Begg *et al.*, 2017; Gurr *et al.*, 2017). This was demonstrated by Letourneau *et al.* (2011)

in a meta-analysis using hundreds of case studies. The message from that and many other studies is that when, for example, flowering plants are added to an agricultural or horticultural system, part of the selection criteria is that their effect on yield should be minimal (e.g. Balmer *et al.*, 2014; Iverson *et al.*, 2014; Pywell *et al.*, 2015; Shields *et al.*, 2016). This has certainly been the case when buckwheat (*Fagopyrum esculentum* Moench.) and phacelia (*Phacelia tanacetifolia* Benth.) are sown between vine rows (Figure 3) (Berndt *et al.*, 2006; Barnes *et al.*, 2009) and when flowering sesame and other species are used in rice (Gurr *et al.*, 2016).



Figure 3. *Phacelia tanacetifolia* in the inter-row of vines, Waipara Valley, New Zealand. Photo: Jean-Luc Dufour.

Plant diversification often positively influences arthropod diversity (Parker *et al.*, 2010; Haddad *et al.*, 2011; Iverson *et al.*, 2014; Hatt *et al.*, 2016; Begg *et al.*, 2017), but also the type of diversification can affect different aspects of the agro-ecosystem, including negative influences. The proportion of natural habitat surrounding the farm directly influences the abundance of natural enemies (Chisholm *et al.*, 2014) but can also increase pest abundance in some cases (Tylianakis & Romo, 2010). This can be attributed to such factors as intra-guild predation, which can include the killing and consumption of potential competitors, i.e. other natural enemy species. Other factors associated with natural habitat that can contribute to increased pest abundance are barriers to movement and resources, such as hedgerows (Mauremooto *et al.*, 1995), as well as abiotic factors (Thomas *et al.*, 1991). These

factors can benefit the second trophic level, i.e. the herbivore pest, more than the third trophic level (the natural enemies).

2.4 Interactions with the landscape

It is important to note that there are many indirect effects that the habitat has on the natural enemy-pest community. Plants can indirectly influence the diversity of parasitoids (parasitic wasps) without affecting pest populations (Tylianakis & Binzer, 2014). Changing land use has also been connected to differing densities of natural enemies (Zhou *et al.*, 2014; Begg *et al.*, 2017) and the presence of secondary pests can influence the abundance of other natural enemies (Bompard *et al.*, 2013). Secondary pests are organisms that do not cause substantial damage unless their natural enemies are removed (Maxwell & Jennings, 1980). Another challenge is that the target pests, or others, can also feed on floral resources and increase their fitness (Gurr *et al.*, 2017). Ecosystem dis-services (EDS) of this type can diminish CBC effectiveness. It has also been suggested that non-crop species can compete for pollinators when the crop requires pollination but actual studies of this are rare (Free, 1993; Holzschuh *et al.*, 2012). These indirect effects on the natural enemy-pest community are likely to be specific for each agro-ecosystem and need to be considered as such when developing habitat management protocols for CBC.

Chemical fertilizers, herbicides, conventional tillage and pesticide application are all primary techniques in agricultural systems. These can stifle agro-ecosystem stability and decrease the abundance and diversity of natural enemies (Altieri, 1999; Begg *et al.*, 2017). Furthermore, moderate shade, adequate labour and appropriate but not prophylactic use of artificial inputs can be combined with ecological engineering (Gurr *et al.*, 2004) to provide high biodiversity and sustainable crop yields (Clough *et al.*, 2011). Many studies confirm that a 'whole system' approach may be necessary to create a sustainable agro-ecosystem (Tscharntke *et al.*, 2007, 2012; Gurr *et al.*, 2017) and that synergies among ecosystem functions may make the system more stable and easier to manage (Iverson *et al.*, 2014; Turner *et al.*, 2014). Specifically, up to 8% of the field production area can be used for wildlife habitat with no negative effect on yields and in most cases where such habitat was present, higher yields were reported, up to 30% in some crops (Pywell *et al.*, 2015). To manage agro-

ecosystems effectively by taking advantage of synergies among ecosystem functions, multiple models may be necessary as they can potentially predict variables accurately enough to be useful in agro-ecosystem management (Turner et al., 2014).

2.5 Conservation biological control in changing climates

CBC manipulates the biotic and abiotic environment to enhance natural enemy efficacy, populations and pest suppression, but a rising concern in the scientific community that can upset CBC functions is changing climates. Evidence is building that suggests that climate change is becoming an imminent global threat to food security (de Schutter, 2010; Tilman *et al.*, 2011; Woodward & Porter, 2016; Myers *et al.*, 2017; United Nations, 2017). One factor is that pests' host range, fecundity and damage potential can increase with the changing climates (Tylianakis *et al.*, 2008; War *et al.*, 2016).

Invertebrates are ectothermic organisms and their physiology and resultant fitness are strongly influenced by microclimate. This makes them very sensitive to climate change at the population level and changes can occur over very short periods of time (Boggs, 2016). In general, a changing climate favours generalist species, which suggests that specialists' populations are likely to decline or become extinct (Hof & Svahlin, 2015; Van Dyck *et al.*, 2015).

Through the above processes, higher temperatures can cause negative impacts on agro-ecosystems. This is because insect outbreaks can increase in frequency due to these changes and associated increased frequency of droughts (Figure 4). Changes in precipitation patterns and increasing of CO₂ in the environment (Murdock *et al.*, 2013; Hof & Svahlin, 2015; War *et al.*, 2016) also impact on invertebrates. For example, an increase in temperature and CO₂ can lengthen the larval period in some species and causes greater mortality (Sharma *et al.*, 2015). High CO₂ levels can reduce populations of the aphid parasitoid, *Diaeretiella rapae* (M'Intosh), by approximately 50% and result in a shorter lifespan for adults (Roth & Lindroth, 1995). Not only does elevated CO₂ decrease natural enemy effectiveness, there can be a difference in the relative effect on predator and parasitoid strategies. Under these conditions, generalists can maintain effectiveness while specialists experience a reduction in fitness (Chen *et al.*, 2005). These CO₂ effects also occur with increased temperature (Hemerik *et al.*, 2015).



Figure 4. A wilting tomato crop during drought in Chitwan, Nepal. Photo: Sundar Tiwari.

Changing climates will also have major impacts on crop production, interactions between plants and invertebrates, and plant physiology. Factors such as temperature, solar radiation, relative humidity, precipitation and wind speed directly influence plant physiological processes (Olesen & Bindi, 2002). Climate affects the phenology of plants, invertebrates and their local abundance and distributions (Hegland *et al.*, 2009). Higher CO₂ concentrations change photosynthesis rates and respiration, and can subsequently impact crop production (Hegland *et al.*, 2009). Similarly, CO₂ and nitrogen enrichment may in some instances increase nectar quality and the abundance of flowers (Hegland *et al.*, 2009). On the other hand, climate changes can reduce plant defences against pests, thereby making them vulnerable to attack (Dhaliwal *et al.*, 2004). Examples of these changes to crops include: rice yields which are lower with increasing night temperatures (Peng *et al.*, 2004), wheat yields that show reductions with temperatures above 32°C (Gregory *et al.*, 1999) and populations of the moth pest, *Helicoverpa armigera* (Hubner), have shifted from tropical to temperate regions where they impact legumes and related crops (Sharma 2005). These plant and invertebrate physiological responses to changing climates lead to shifts in geographical distributions, changes in food availability and increased competition among organisms. With these changes and the warming of the environment, fragile niches could be destroyed and the resulting agro-ecosystems are likely to have reduced natural enemy diversity with increased pest outbreaks and successful invasions as a result.

2.6 Multiple ecosystem services

CBC is one of many ES, including pollination, nutrient cycling and soil aeration among others. ES are processes and functions derived from nature that benefit humans directly or indirectly (Bennett & Chaplin-Kramer, 2016; Costanza *et al.*, 2017). Multiple ES can arise from the deployment of SNAP (see Section 1) and other agri-environment interventions but have been greatly undervalued (Sandhu *et al.*, 2013). Although they can lead to many benefits beyond those originally intended, these are rarely quantified. For example, phacelia can enhance biological control but is also greatly favoured by bees (Sprague *et al.*, 2016). Furthermore, endemic New Zealand flowering plants in vineyards (Figure 5) can enhance multiple ES such as weed suppression, mineralization of plant material and soil water retention while leading to increased natural enemy abundance and efficacy (Shields *et al.*, 2016).

However, this is not the norm as the CBC literature has been largely limited to single ES studies which ignore the other ES, heavily restricting end-user attractiveness and adoptability. These single ES studies range from field research on natural enemy-pest communities and floral resources (Scarratt *et al.*, 2008; Gurr *et al.*, 2012) to laboratory studies looking at natural enemy flower preferences (Sivinski *et al.*, 2011), oviposition rates and longevity when they are fed nectar from different sources (Lee & Heimpel, 2008). While work on these specific aspects of CBC are helpful in understanding parts of the full system, recent research suggests that designing protocols that potentially enhance multiple ES would have a higher value and possibly a greater likelihood of adoption (Crowder & Jabbour, 2014; Iverson *et al.*, 2014; Geertsema *et al.*, 2016; Gurr *et al.*, 2016). For instance, landscape-scale manipulations can be successful in this respect (Tscharntke *et al.*, 2007; Rusch *et al.*, 2016) but can sometimes lead to results which are not related to the original aim (Jonsson *et al.*, 2012).

An example of highly successful enhancement of multiple ES is the 'push-pull' work in East Africa (Khan *et al.*, 2000). This economically viable approach has been adopted by over 30,000 farmers and provides multiple ES such as pest management, soil fertility, nitrogen fixation and animal fodder. These ES provide farmers with multiple avenues of income with low inputs such as increased maize yields (by 2.5 t/ha) and sorghum yields (by 1 t/ha), as well as higher milk production (Khan *et al.*, 2011). The integration of multiple ES in agro-ecosystems with approaches such as 'push-pull' is considered by many to be a key component of future food production (de Schutter, 2010; Khan *et al.*, 2011; Tscharntke *et al.*, 2012; Iverson *et al.*, 2014; Gurr *et al.*, 2016; Costanza *et al.*, 2017; Gurr *et al.*, 2017).



Figure 5. An endemic New Zealand plant (*Hebe chathamica* Cockayne et Allan) under vines, providing multiple ecosystem services to the vineyard. Photo: Jean Jack.

2.7 Ecosystem dis-services

Habitat manipulation intended to enhance CBC can have specific unintended negative impacts; these are known as ecosystem dis-services (EDS) (Zang *et al.*, 2007; Gurr *et al.*, 2017). For instance, added vegetation such as floral resources (i.e., SNAP) may benefit pests more than the natural enemies (Lynch *et al.*, 2001; Brimner & Boland, 2003; Winding *et al.*, 2004; Tscharrntke *et al.*, 2005; Carvalho, 2006; Zehnder *et al.*, 2007; Cullen *et al.*, 2008; Wolfenbarger *et al.*, 2008; Roubos & Liburd, 2009). An example is that the fecundity of the moth pest, *Epiphyas postvittana* (Walker), is enhanced with an

increase in the availability of some flowering plants (Begum *et al.*, 2006). This indicates the importance selecting plant species that minimize EDS which could be in the form of competition for resources (Gurr *et al.*, 2017) and pollination (Holzschuh *et al.*, 2012) between the added vegetation and crop species. Also the added vegetation could be allelopathic towards the crop (Zhang *et al.*, 2007). Furthermore, the wider complexities of food webs need to be considered, for instance honeydew-producing pests such as mealybugs are ‘farmed’ by ants that are predators of many natural enemies of the mealybug and may reduce biological control of this pest (Daane *et al.*, 2007). Many non-crop plants are also hosts of these (Gutierrez *et al.*, 2008) and other pests which complicates the selective process for vegetation to be used in CBC habitat manipulation (Winkler *et al.*, 2010).

Potential EDS can be reduced by considering them when designing CBC experiments such as measuring *E. postvittana* development on floral and non-floral plant tissues while investigating multiple ES (Shields *et al.*, 2016). Furthermore, modelling the key parameters involved in natural enemy traits can be employed to inform biological control (Kean *et al.*, 2003). Complex modelling could be used to develop sophisticated CBC service providing protocols (SPPs) (Wratten *et al.*, in press) that prevent EDS. However, to achieve this, substantial knowledge of how habitat management practices reduce pest damage and what are the associated EDS need to be determined (Gurr *et al.*, 2017).

2.8 Implementation of conservation biological control: From outputs to outcomes

One of the largest barriers to implementation of CBC research results to farming practices is relaying information to end-users (primarily farmers) and their willingness to uptake and deploy that knowledge. A specific barrier that reduces uptake of this is the perceived negative impacts on the livelihood of the local community when CBC advice is provided ‘remotely’, for example at government level (Bennett & Dearden, 2014). The solution to this is improved communication between policy-makers, scientists and farmers, and improved farmer education about CBC (Bennett

& Dearden, 2014; Murage *et al.*, 2015; Wyckhuys *et al.*, 2017). This may be achieved using information and communication technologies (ICT) which are increasingly being used with hand-held devices such as tablets and mobile phones that can provide multi-media access to CBC information with video, SMS and voice-based information delivery (Aker, 2011; Wyckhuys *et al.*, 2017). These can be two-way information delivery systems where farmers and growers can communicate issues to local government (Aker, 2011; Vong *et al.*, 2013). For example, in Vietnam, rural telecentres have been implemented to provide technologically isolated agricultural communities with access to a two-way information channel that provides information and solutions to problems that local farmers have conveyed (Vong *et al.*, 2013). These ICT communication pathways are still largely undervalued, but have enormous 'penetration' potential, particularly where other infrastructure is underdeveloped. However, actual adoption of CBC through these delivery systems is still limited (Aker, 2011; Wyckhuys *et al.*, 2017).

Although modern 'delivery systems' such as information on social media can be used, and probably have more 'penetration' than leaflets and farmer meetings etc., these need to complement direct 'pathways to implementation' involving on-farm, face-to-face communication. Trends in social science research in agriculture suggest that working with the famers directly, including having demonstration or trial plots, can be successful (Estrada-Carmona *et al.*, 2014). This needs complementary approaches as well, including farmer field schools (Figure 6), led not by scientists but by 'farmer teachers' which are direct and proven pathways to implementation (Warner, 2007; Khan *et al.*, 2011; Ferguson & Lovell, 2014; Kiptot & Franzel, 2014; Waddington *et al.*, 2014; Wyckhuys *et al.*, 2017).



Figure 6. A farmer field school in action in a rice crop in Birendranagar, Nepal. Photo: Sundar Tiwari.

2.9 The future of conservation biological control

Many international agencies are indicating that modern, high-impact farming, with associated dependence on fossil fuels and a high level of external costs (negative impacts on human health and the environment) cannot continue (de Schutter, 2010; Sandhu *et al.*, 2012; Tscharncke *et al.*, 2012; Pywell *et al.*, 2015; Gurr *et al.*, 2016; United Nations, 2017). For example, the 'Green Revolution' was a 'solution' to higher yields (Gaud, 1968). However, it became out of favor because of its negative socio-economic effects and the external costs associated with its adoption and practices (Pearse, 1980; Tilman, 1998; Iverson *et al.*, 2014). Now, potentially oxymoronic ideas such as 'sustainable intensification' are frequently advocated (e.g. Godfray *et al.*, 2010; Pywell *et al.*, 2015; Gurr *et al.*, 2017). Whatever the proposed improvements, CBC needs to be part of a paradigm change in terms of how agriculture is practised, with a much greater emphasis on enhancing the contribution that ES

can make to sustainability, including reduced dependence on fossil-fuel based inputs. The key challenges will be: (1) understanding the food-web dynamics of which CBC is a substantial part; (2) establishing clear pathways from a research outputs to achieving real outcomes; and (3) moving from high-level policy goals to practical on-farm changes. The latter is made more difficult by the relentless pursuit of GDP as part of economic growth (Costanza *et al.*, 2014) coupled with key governments pursuing neo-liberal policies in which interventions in society by the state are transferred to private enterprise. Compounding the above is the intense and well-funded marketing by agro-chemical companies, supporting prophylactic use of their products, coupled with a low educational standard in developing countries. Converting science outputs to outcomes, including the deployment of proven pathways to implementation remains a key challenge in CBC, and in all agro-ecological approaches.

Chapter 3

A comparison of anesthesia techniques for entomological experimentation: Survival of the leaf-mining fly pest *Scaptomyza flava* Fallén (Diptera: Drosophilidae)

3.1 Introduction

A key pest of brassicas worldwide is the leaf-mining fly, *Scaptomyza flava* (Fallén, 1823), which can cause cosmetic damage leading to crop rejection by supermarkets/consumers (Shakeel et al., 2009). In climates where leafy salad brassicas are harvested all year, the flies are almost always present (Seraj, 1994). This necessitates control throughout the entire harvesting season and usually consists of the prophylactic application of insecticides. One way of ameliorating the negative environmental impacts of this approach is to enhance the effectiveness of biological control by providing alternative food sources for natural enemies. The planting of selected flowering plants can be useful in this respect, as many parasitoids and other insects feed on certain nectars. This, in turn, can improve their efficacy (Tylianakis et al., 2004; Gurr et al., 2012) by increasing fecundity and survival of natural enemies (Gurr et al., 2017). Handling of live insects is necessary to evaluate different flowering plants for their potential as a food source. This is difficult without anesthesia.

Documented experiments began in the 1920s to investigate possible techniques to render insects immobile without killing them (Willis, 1925). Many approaches have been developed since then, each with its own advantages and disadvantages (Wedberg & Clarke, 1947; Worthen & Moore, 1991; Ratterman, 2003; Chen & Hillyer, 2013). For example, ether can be an effective anesthetic but is harmful to the user, whereas chilling is benign to the user but has a short anesthesia effect (Barron, 2000; Ratterman, 2003). If the intention is to work on the behaviour, ecological fitness or other aspects of an insect's biology, it becomes important to know how these approaches will affect it. Therefore, alternative techniques that can be managed so as not to harm the user or the insect are needed (Champion De Crespigny & Wedell, 2008; Cooper, 2011; Smith et al., 2014). Three types of anesthesia have recently been used with varying success. These are carbon dioxide (CO₂), chilling and triethylamine vapour (TEA). Here, we compare these three approaches, because of the possibly unjustified popularity of both CO₂ and chilling, and the relatively unknown sub-lethal effects of TEA. This compound is used in a commercial product initially developed for anesthesia of *Drosophila* spp. for educational purposes, but little work has been carried out to elucidate its physiological mechanisms of action and lethal and sub-lethal effects on insects (Ratterman, 2003). This study focuses on survival as the key 'fitness' trait measured because this is the variable that is most usually

quantified in laboratory work on developing agro-ecological schemes (Berndt et al., 2006; Nafziger & Fadamiro, 2011). TEA is suspected to be a superior anesthesia technique as it has commercial uses that tout its safety, ease, speed and duration of anesthesia (Supply, 2012; Binkley, 2016). We used the common leaf-mining fly pest, *Scaptomyza flava* (Fallén), as our study organism.

3.1.1 Carbon dioxide as an insect anesthetic

CO₂ has been used for decades for this purpose, documented as early as the 1920s (Willis, 1925). At high concentrations, this gas interferes with signals that trigger central nervous system function, and can stimulate some behaviour (e.g. foraging for food) at low concentrations in some insects (Nicolas & Sillans, 1989; Badre et al., 2005). Due to its ease of use, reproducible results and relative safety for humans, CO₂ has often been employed as the primary insect anesthesia technique (Nicolas & Sillans, 1989; Badre et al., 2005; Champion De Crespigny & Wedell, 2008). However, care must be taken when using this gas, as it can also have adverse effects on insect behaviour and fertility (Ribbands, 1950; Nicolas & Sillans, 1989; Champion De Crespigny & Wedell, 2008). Such effects can occur across many insect orders, from survival and fecundity in orthopterans (Chen et al., 2013), to role-switching in social insects such as honey bees (Nicolas & Sillans, 1989). In the Drosophilidae, which includes *S. flava*, CO₂ affects *Drosophila* species' fecundity, survival (Barron, 2000; Fresia et al., 2001; Champion De Crespigny & Wedell, 2008), metabolic processes (Colinet & Renault, 2012) and learning/memory (Margulies et al., 2005). Another drawback with this gas is that it must be stored under pressure, presenting possible safety issues for the user because of the weight of the cylinders, which can lead to injury (Artiss & Hughes, 2007). Depending on duration of use, rental/purchase costs can become high because of the equipment needed for application and storage.

3.1.2 Chilling as an insect anesthetic

As with CO₂, chilling has been commonly used as an insect anesthesia technique for many years because of its ease of use and safety (Wedberg & Clarke, 1947; Nilson et al., 2006; Champion De Crespigny & Wedell, 2008). It allows the user to take advantage of triggering insect chill coma, which is a threshold at which the neuromuscular activity comes to a halt at low temperatures (MacMillan & Sinclair, 2011). However, results are variable as they depend on the environment from which the insects were collected or reared and the insect species' response to cold stress. For example, some insects from tropical regions have less tolerance of temperature fluctuations than do those in more temperate climates (David et al., 1998; Barron, 2000; Reynolds & Orchard, 2011). Some experiments have successfully used chilling alone (Reynolds & Orchard, 2011), while one had to use CO₂ in conjunction with chilling to increase survival after recovery from cold exposure (Nilson et al., 2006). Additional effects reported from chilling have been disruptions to mating behaviour and genetic upregulation. Condensation on the wall of the container can damage insect wings, which are used in

mating displays in some insect groups (Artiss & Hughes, 2007). Also, copulation latency after recovery has been observed (Barron, 2000). This suggests that exposure to low temperatures may affect reproduction in a range of ways. When observing genetic changes, upregulation of genes in response to cold stress has been observed in *Drosophila melanogaster* Meigen (Zhang et al., 2011) especially if it has had repeated exposure. This leads to differences in physiological responses, such as increased cold tolerance, which also occurs during acclimation experiments (Colinet et al., 2012; Marshall & Sinclair, 2012).

3.1.3 Triethylamine as an insect anesthetic

Common use of TEA began in the early 1990s, mostly involving *Drosophila* spp. (Worthen & Moore, 1991; Kauffmann et al., 1995). Subsequently, this compound has been used to facilitate the observation of anatomical structures (e.g. viewing the heart and spiracles) inside living dissected insects (Vogler & Ocorr, 2009; Boppana & Hillyer, 2014). The generally accepted disadvantage of this compound is its volatility (Ratterman, 2003; Artiss & Hughes, 2007), which could lead to acute toxicity in humans if it is handled inappropriately. To mitigate this, appropriate handling procedures and personal protective equipment are necessary. However, this compound can be used in small diluted quantities, as only a drop is needed for anesthetizing an entire 35 ml vial of insects (Fresia et al., 2001). A 1 L bottle of TEA costs approximately 20USD so, when diluted, its cost is minimal and approximately 250 ml was used for this study. This makes TEA cost effective, easily transportable (because only small quantities are needed) and reduces risks to users.

3.2 Materials and Methods

S. flava was kept in colonies in 60x60x60cm BugDorms (<http://bugdorm.megaview.com.tw/>) and reared on trays of *Brassica juncea* (L.) Czern 'Mizuna' seedlings (Appendix, Figure A1), which were kept at 22°C ±2°C in controlled temperature rooms at the Bio-Protection Research Centre, Lincoln University, New Zealand. The Mizuna was made available to the flies when it was 3 weeks old. To select flies for the experiments a colony was chosen where the adults were removed after a week of exposure to the plants, allowing subsequent cohorts to be used for experiments (i.e. 24hr old flies were used in each experiment).

This study comprised three groups of experiments. A preliminary range-finder assessment was used to find a range of rates to test from each anesthesia technique. Once that range was found another experiment (Group 1) was used to find the most appropriate way to administer each anesthesia technique on its own and lastly, Group 2 was conducted to determine whether CO₂, chilling or TEA were the best anesthesia technique overall. Once the rangefinder was completed the Group 1

experiment was carried out. Once the best rates were selected from this experiment (Group 1) for each anesthesia technique, Group 2 was conducted.

Recovery and number dead after 24 h were the response variables that were evaluated to discover which rates within anesthesia agents (Range finder and Group 1) were best as well as finding out which anesthesia agent was best overall (Group 2). The group 2 experiments also observed longevity. Recovery of a fly was defined as its ability to fly when disturbed (i.e., in response to the container being gently tapped or in response to user movement). Only three replicates were carried out (see 3.5 Discussion) as a power analysis predicted that too many replicates would have been needed to achieve ideal resolution in the analyses (Table 3.1).

Each anesthesia technique used different variables (e.g. temperature, concentration) because each had a different method of application. Recovery time and mortality were compared between rates of the same anesthesia technique for the Group 1 experiment and between anesthesia technique treatments in Group 2. In Table 1, the threshold recovery time (900 s or 15 min) was selected as a compromise between other options. This was because CO₂ recovery time was measured in seconds, whereas that for TEA was hours.

Table 3.1 Power analysis for the experiments that observed recovery time between rates of each anesthesia technique (Group 1). Threshold recovery time (15 min) was the variable that determined the number of replicates required.

Anesthesia technique	Pooled SD	Threshold recovery (min)	t-value (95% conf)	Degrees of freedom	Replicates required
CO ₂	1418.15	15	2.015	20	72,584
Chilling	339.21	15	2.015	44	4,153
Triethylamine	20.36	15	2.015	185	15

3.2.1 Range finders

These three anesthesia methods have been described in the literature, but none has used *S. flava* nor compared all three together. Using several previous studies as guidelines, a series of preliminary experiments was carried out (range finders) to find the range of the variables in which each anesthesia technique could be tested. As each anesthesia method has different properties, different variables were measured for each one. CO₂ used only duration of exposure because concentration could not easily be measured/changed and most published papers do not report concentration used when applying CO₂ (Nicolas & Sillans, 1989; Nilson et al., 2006; Colinet & Renault, 2012). The exposure durations were chosen at an exponential rate starting from 5 s. Chilling used duration of exposure and temperature. The times and temperatures were selected by using several earlier studies as a guide (David et al., 1998; Nilson et al., 2006; MacMillan & Sinclair, 2011; Reynolds &

Orchard, 2011). Lastly, TEA durations of exposure were selected ad-hoc, as all publications found had used a formulated version (Ratterman, 2003; Artiss & Hughes, 2007; Chen & Hillyer, 2013; Boppana & Hillyer, 2014) of TEA (FlyNap®, 50% TEA) with which the user had previous experience (<3 min are needed for complete anesthesia). The concentrations used in the range finder were 50% and 100% because the commercial product uses a 50% solution and based on previous experience with this compound. 25 flies were randomly aspirated from the colonies according to the above stated methods

3.2.2 Group 1 - Optimal treatments experiment

Controls for Group 1 were ambient conditions for CO₂ and chilling but 100% ethanol for TEA. The latter was chosen because it was diluted in ethanol for experiments. Ethanol did not affect fly recovery or mortality rates when compared with the other controls (ambient conditions). 25 flies were chosen according to the above method for each treatment for each of the anesthesia techniques.

Carbon dioxide

After the range-finder was completed, 10 periods (5, 60, 600, 900, 1200, 1500, 1800, 4800, 6000 s and control) were selected and replicated three times each (see above). Some of the time periods used in the range finder were changed in this experiment to account for the inclusion of observation periods. The exposure times of 10240 (approx. 170 min) and 20480s (approx. 341 min) had initially been investigated but were removed because they were too time-consuming and expensive to carry out. CO₂ was applied by inserting a tube into a LabServ P35 35ml vial sealed with a foam plug (Figure 3.1a). A steady stream of CO₂ that was gentle enough not to move the flies around in the vial was used. Then flies were sexed under a dissecting microscope with 16x magnification and moved to new vials, each of which contained an Eppendorf tube filled with water and plugged with cotton (Figure 3.1c) so the flies could re-hydrate. The Eppendorf tube was fixed to the side of the vial to protect anesthetized flies from being crushed by possible movement of the tube when vials were set on their side to be monitored in the controlled temperature room (Appendix A, Figure A2). These treatments were checked every 5 min for 6h or until all flies recovered; any deaths were recorded after 24 h.

Chilling

15 temperature/exposure combinations (0°C, 2°C, 4°C and 2 h, 4 h, 8 h, 16 h, and 24 h) were chosen with 3 replicates each. Into each vial, 25 flies of unknown sex were placed. The vials were then sealed with foam plugs (Figure 3.1a) and the evaluation was carried out by placing the vials in holes in a Styrofoam float so that they were in the liquid. Temperature was managed by a temperature-controlled water bath (±0.5°C) using a Low Temperature Circulator LTD-6 (Grant Instruments Ltd, Cambridge, UK). There was very low (unmeasurable) temperature variation in this water-bath (10 L

60% propylene glycol, 40% water) method; a refrigerator would not have had such temperature stability. When the targeted exposure had been reached, the vials with the flies were removed and each batch of flies was transferred to a new vial. The latter contained a water-filled Eppendorf tube and was then monitored in the controlled temperature room as noted above and shown in Figure 3.1. The flies were monitored every 5 min for 6 h. 24 h after this period, mortality was recorded.

Triethylamine

There were thirty time/concentration combinations evaluated (10, 20, 30, 40, 50, 60 s and 100%, 75%, 50%, 20% TEA in ethanol) with three replicates for each combination. These treatments used a cotton swab soaked in TEA solution was inserted into the vial through a plastic drinking straw, which was then sealed with a foam plug (Figure 3.1a) at the termination of exposure. After duration of exposure was met, the straw and swab were removed and the flies were gently knocked down to the bottom of the tube until they were fully anesthetized. A new cotton swab was used for each application of TEA. Mortality was recorded as above.

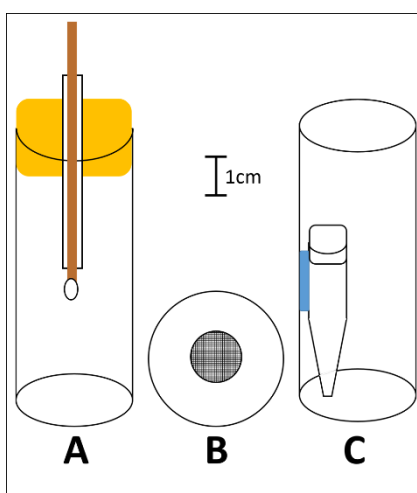


Figure 3.1 Foam-plugged 35ml vial with straw and swab inserted for application of triethylamine (A), meshed cap (B) Eppendorf tube filled with water and with a cotton-wool plug. A similar vial setup but no swab and straw was used when applying CO₂. The vial with a foam plug (A) was floated in a water bath for the chilling treatments. All treatments used the vial depicted in C for data collection.

3.2.3 Group 2 - Final comparison experiment

The experimental design allowed for the optimal rates from each of the three anesthesia techniques described above to be determined and included a control. The control was ambient conditions because Group 1 found that ethanol had the same response as ambient conditions. The criteria for selecting the optimal treatment from each anesthesia approach was based on meeting the 900s (15 min) threshold for sexing, the longest anesthesia period and fewer deaths after 24 h. The experimental arrangement was a randomized block design with three replicates. Twenty flies were aspirated into a 35ml tube that were sexed to get as close to a 1:1 sex ratio as possible. Sexing was done using a magnification loupe before aspiration and were confirmed after immobilized using a dissecting microscope. The sexes were then put into separate vials and monitored for recovery (Appendix, Figure A2). Survival was recorded every 24 h after recovery until all flies had died. These methods were derived and adapted from other studies that also evaluated invertebrate anesthesia methods (Perron et al., 1972; Barron, 2000; Champion De Crespigny & Wedell, 2008; Chen et al., 2013).

3.3 Analysis

As the range finders were run only once to determine a range for selecting the experimental variables, i.e. survival/recovery times, no statistical analysis was done. For subsequent tests, Shapiro-Wilks tests of normality showed that all data were non-normally distributed (all p-values < 0.001) so non-parametric analyses were needed.

3.3.1 Group 1 analysis

Each anesthesia technique may be administered in different ways, which changes the variables tested. The goal in Group 1 was to find the most appropriate way to administer each anesthesia method on its own. For each of these experiments, recovery time and mortality rate were estimated. A Kaplan-Meier estimator was used to determine the median recovery time in each treatment group. However, there was a problem with this method. Some parameter estimates diverged to + or – infinity, a phenomenon known as monotone likelihood which renders parameter estimates inaccurate (Heinze & Schemper, 2001). When the Cox's Proportional Hazard model experiences monotone likelihood, the resulting estimates become arbitrary and inaccurate (Heinze & Ploner, 2002). To resolve this problem, Cox's Proportional Hazard model was used with Firth's penalized likelihood (Heinze & Dunkler, 2008). In each experiment, the different model structures were compared to find the best representative. Each treatment was then tested to determine if there was a significantly different recovery time.

How the death rate changed with treatment was also estimated. As with recovery time, a Kaplan-Meier estimator and Cox's Proportional Hazard model were used. After analysing the results from the recovery and death estimates in Group 1, a single treatment was identified in each anesthesia type (e.g. CO₂, chilling, and TEA) which had the longest recovery time and fewest deaths in 24h. The chosen anesthesia treatments were used in a subsequent experiment in group 2. P-values were reported from each of the likelihood ratio tests from the model comparisons.

3.3.2 Group 2 analysis

The goal in group 2 was to determine whether CO₂, chilling or TEA was the best anesthesia technique overall. To do this, a single experiment comparing the best treatments identified for each anesthesia in Group 1 was performed. A control where no anesthesia was administered was also included. The recovery time and survival for each anesthesia technique was estimated using the Kaplan-Meier estimator and Cox's Proportional Hazard model with and without Firth's correction (noted when used). For this experiment, P-values from the likelihood ratio tests were reported. The median recovery and median deaths with their respective confidence limits were also reported. To complete all analyses in this work, R studio (R version, 3.3.1) with the 'survival' (v.2.38, Heinze & Ploner, 2016) and 'coxphf' (v.1.12, Therneau & Grambsch, 2000) packages were used.

3.4 Results

3.4.1 Range finders

The results in Appendix A, Table A.1 were used to select the exposure times/concentrations/temperature for each anesthesia type, respectively. For CO₂, exposure durations after 1800 s had a spike in the number of deaths and used up CO₂ supplies rapidly. The canisters were completely exhausted after one or two treatments so tests longer than 1800 s were not used. Next, chilling did not show much variation between treatments so an exponential increase in the exposure time was used at each of the three temperatures, starting at 2 h exposure. This led to a final evaluation of 0°, 2° and 4°C with exposure times of 2, 4, 8, 16 and 24 h (120, 240, 480, 960 and 1440 min, respectively). Lastly, two TEA treatments were carried out, but all the flies died at or shortly after 60 s exposure. This led to the development of the design that used 25, 50, 75 and 100% TEA with ethanol as the solvent. Each of these concentrations was then paired with 10, 20, 30, 40, 50 and 60 s exposures, making 24 concentration/time concentrations.

3.4.2 Group 1 - Optimal treatments

Carbon dioxide

The likelihood ratio test suggests that the treatments significantly differed ($P < 0.0001$). The treatments below 900 min exposure (5 min, 60 min, and 600 min) were removed from selection

because they did not meet the 900 s (15 min) recovery threshold (Appendix, Table A.2). CO₂ exposure at 1500 s was selected because it was the treatment with the second highest recovery time but had fewer deaths than the 1800 s exposure treatment (0 vs ~3, respectively).

Chilling

The likelihood ratio tests for the different chilling models suggest that there is no interaction effect of exposure time with temperature ($P = 0.8393$). However, different temperatures do significantly ($P < 0.0001$) affect the recovery time of the flies. Although temperatures do affect the recovery time, none of the temperature treatments met the 900 s (15 min) threshold (Appendix, Table A2). The longest recovery time occurred when flies were exposed to 0°C for 24 h, but because exposure times did not significantly affect the recovery time, 0°C at 8 h exposure was selected for convenience.

TEA

The likelihood ratio tests for the different TEA treatments (concentration and exposure time) suggested that there is an interaction with fly sex (25%, $P = 0.0028$; 50%, $P = 0.0135$; 75%, $P = 0.0045$; 100%, $P = 0.002$). This suggests that males and females respond differently to TEA treatments (concentration and exposure time) and that females generally recover sooner than males ($P < 0.0001$). The flies in all treatments exceeded the recovery time threshold, but 25% and 100% TEA were removed from the selection. 25% TEA treatments were removed because the flies still retained mobility, albeit highly reduced, making sexing and transferring between vials difficult. The 100% treatment, on the other hand, had a full anesthetic effect for well over 2 h but they caused more deaths than the other treatments (4 for 100%, 2 for 75% and 0 for the rest). Using the longest anesthetic effect and the lowest number of deaths as the selection criteria led to the decision that 75% TEA at 60s was the optimal candidate.

3.4.3 Group 2 - Final comparison experiment

The Cox's Proportional Hazard model demonstrated a significant difference between the fly sexes ($P < 0.0001$), so all results in Table 3.2 are separated by sex. There were also clear significant differences between all treatments for recovery times (Figure 3.2 and Table 3.3), with TEA having the longest recovery (145 and 105 min) and chilling having the shortest (5 min). Lastly, the survival of all treatments were not significantly different from each other (Figure 3.3 and Table 3.3).

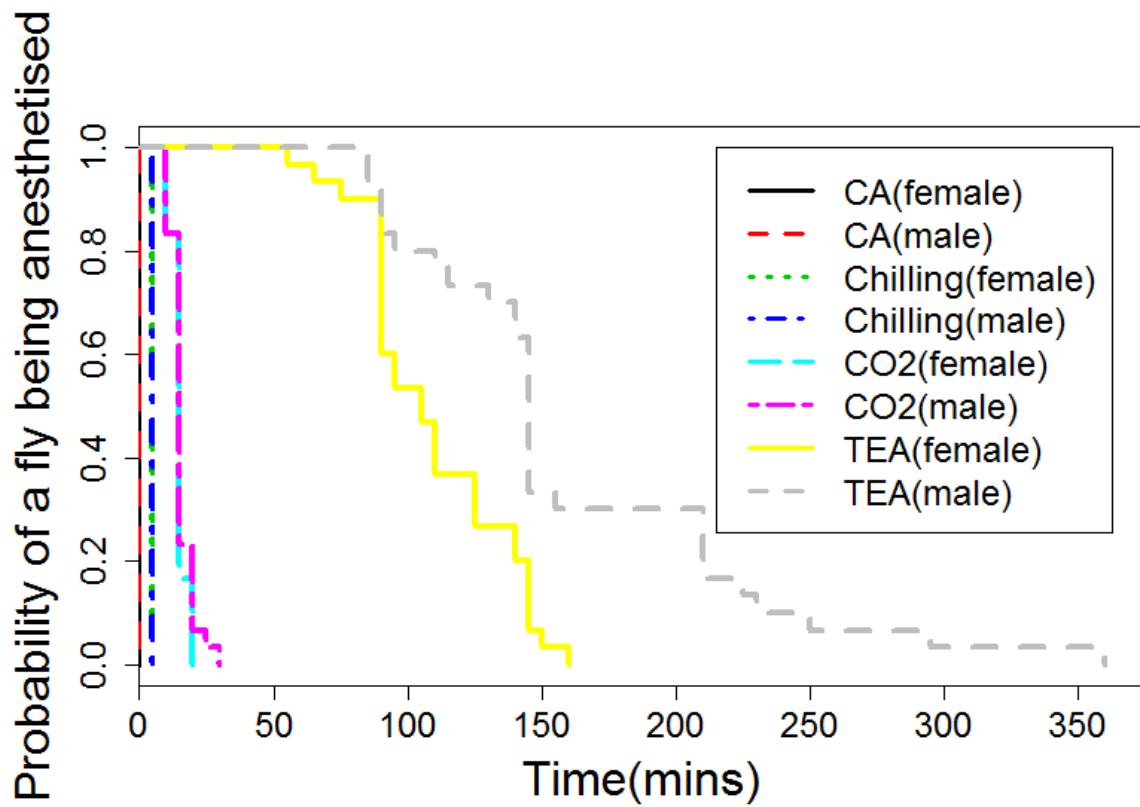


Figure 3.2 Kaplan-Meier analyses of time to recover from anesthesia techniques. The curves for the sexes are similar for CO2 and chilling but different in triethylamine (TEA). The graph for controls is not shown as it would be superimposed on the y-axis.

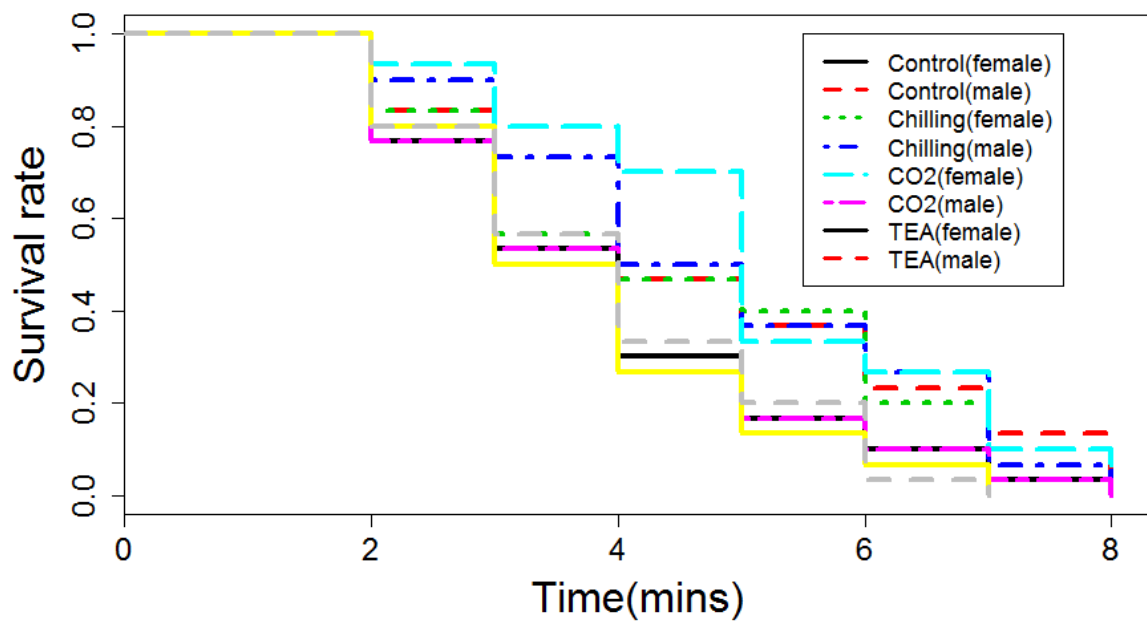


Figure 3.3 Survival rates of *Scaptomyza* in the three anesthetic treatments and the control. TEA = trimethylamine.

Table 3.2 Results from comparing the three anesthesia techniques (Group 2 experiment) showing the estimates for the median recovery and median deaths with their respective lower and upper confidence limits. The anesthesia treatment chosen is highlighted in bold. Two treatments are selected because anesthesia type, not effect was the category of interest. The highest median recovery time was used to determine the best treatment because there was no significant difference between the longevities across all treatments.

Anesthetic	Intensity	Exposure	Median recovery (min)	Lower 95% CL (min)	Lower 95% CL (min)	Median deaths	Lower 95% CL (deaths)	Upper 95% CL (deaths)
Control (F)	NA	NA	0	NA	NA	4	3	5
Control (M)	NA	NA	0	NA	NA	4	3	6
CO ₂ (F)	NA	1500s	15	15	15	5	5	6
CO ₂ (M)	NA	1500s	15	15	15	4	3	4
Chilling (F)	0°C	8h	5	5	NA	4	3	6
Chilling (M)	0°C	8h	5	5	NA	4.5	4	6
TEA (F)	75%	60s	105	90	125	3.5	3	4
TEA (M)	75%	60s	145	140	210	4	3	5

All estimates are based on Cox's Proportional Hazard model without Firth's correction because infinite values were calculated with Firth's correction and can not be evaluated. 95% CL = 95% confidence limit of the mean; F = female; M = male.

3.5 Discussion

The work in this chapter should be of value to researchers in experimental entomology because the relatively depauperate literature in this area could lead experimenters to use approaches with limitations or risks unknown to them. Supporting this view is the fact that an unpublished pre-print of an earlier version of this chapter in PeerJ has already attracted many views (Appendix, Figure A3). Comparing these approaches to insect anesthesia has shown that each anesthesia technique affects the flies' recovery differently. This also demonstrates that there may be different uses for each of these anesthesia techniques. For example, when examining the physiology of an insect while it is alive, TEA would be favoured because of its long duration of effect after application (Vogler & Ocorr, 2009; Chen & Hillyer, 2013; Boppana & Hillyer, 2014). If transferring the flies from one container to the next is the only action desired (Ratterman, 2003; Artiss & Hughes, 2007), then all three would be suitable. In cases where a short recovery time is needed, TEA is not ideal; in all other cases, it is at least equitable as an appropriate anesthesia technique when compared to the other two. Although three replicates is unusually low, significant results were still found. To complete even this low replicate number took 6 months and more would have been logistically impossible. Similar work on

insect anesthesia techniques has also used a low replicate number, e.g. Perron et al., 1972, Barron, 2000 and Champion De Crespigny, 2008.

For this experiment, we chose TEA as the optimal anesthetic because of its ease of application, cost effectiveness, duration of anesthesia effect and because the length of recovery time was not an important factor in further experimental designs. Results here have shown that using CO₂ or chilling can be ineffective when trying to perform observations with anesthetized insects within a specified time interval (e.g. less than 900 s did not allow for sexing), despite their frequent use (Perron et al., 1972, Smith et al., 2014). This work therefore leads to new possibilities for insect anesthesia, when killing the insect target is often undesirable (Oi et al., 2013; Price et al., 2015; Cheng & Lin, 2016; Sikulu-Lord et al., 2016). Other than the effects on survival, other potential sub-lethal effects of TEA were not investigated in this study, whereas these effects have been thoroughly investigated with the other two anesthesia techniques. The unforeseen effects that TEA could have include, but are not limited to, changes in fecundity, host-searching, and memory and flight patterns (Voinovich et al., 2012; Chen et al., 2013). It is also important to reiterate that this compound does have negative human health consequences if proper precautions are not followed (Sciencelab, 2013), but it seems that a lower concentration (50%) could be used to lessen the hazards. However, the other two methodologies can also have detrimental effects if handled improperly, especially CO₂; accidents with heavy cylinders, for example. It is unlikely, however that the chilling technique used here will negatively impact on the user.

Chapter 4

Inter- and intra-specific comparisons of plant nectar on the relative longevities of the fly pest *Scaptomyza flava* Fallén and its parasitoid, *Asobara* sp. (*nr. persimilis*) Papp

4.1 Introduction

Flowering plants are suitable candidates in programmes for conservation biological control as they can enhance this and other agroecosystem services (Fiedler et al., 2008). Shelter, nectar, alternative food and pollen (SNAP) is a concept that summarises many of the benefits that flowering plants can provide to an ecosystem (Gurr et al., 2017). Many other practices, such as ecological enhancement schemes which include pollination have flowering plants integrated into them because of these benefits (Wratten et al., 2012; Sprague et al., 2016). When SNAP is provided in an ecosystem, it provides a reservoir of resources that can be drawn upon by the organisms to improve their ‘fitness’ (Nilsson et al., 2016). These allochthonous resources are sometimes also called ‘resource subsidies’ (Richardson et al., 2010). This is especially important in agroecosystems which are depauperate in non-crop habitats (Wratten et al., 2013). Such landscapes often lead to high pest populations and this results in increasing pesticide use. These have external costs (to human health and the environment) (Bourguet & Guillemaud, 2016).

Such costs have shifted public opinion to some extent towards a demand for low to no residues from pesticide applications on or in consumed products. This demand has created a need for alternatives to pesticide use. Classical biological control, in which potential agents are imported to manage an introduced pest, has been investigated for decades as an alternative and has had successes (Gurr et al., 2012). However, with tightening border bio-security, it is becoming more and more difficult to import such organisms, especially in New Zealand (Eschen et al., 2015). Another alternative approach is conservation biological control. This manipulates the cropping environment to improve the ‘fitness’ and efficacy of existing natural enemies of the pests. Generally, no importation is required in this approach, making it more easily accessible to and accepted by growers, the public and regulatory organisations.

Globally, leafy greens are an important part of human diets, especially in south and east Asia, and elsewhere are grown for packaged salads for supermarket sales. In New Zealand, salad crops generated \$NZ 53.7m (~\$US 37.8m) during the 2015-2016 year (Wilkinson et al., 2015). In this system, most crops are brassica species grown for their leaves, which are harvested ~21 days after

sowing, then processed for packaging. The major pest on these brassicas in New Zealand is *Scaptomyza flava*, Fallén (Drosophilidae) a leaf mining fly, which is currently controlled by prophylactic pesticide applications. *S. flava* larvae mine the leaves and cause cosmetic damage. If these damaged leaves are not found during processing, the entire product can be rejected by the market; there is a zero tolerance for this type of damage. This suggests that there is no scope for biological control but if the proportion of leaves attacked is reduced, this would lead to a lower rejection rate in the packhouse, saving labour and time (and thus money). The parasitoid species evaluated in this study was *Asobara* sp. (*nr. persimilis*) Papp, 1977 (Braconidae). This is a koinobiont endoparasitoid that targets the larval and pupal stages of *S. flava* and *Asobara* sp. (*nr. persimilis*) originates from Australia (Prince, 1976) which this species is known to be near if not this exact species (robust analysis is still needed to find a conclusion). It was chosen because it had been released across New Zealand to control *S. flava* (MacDonald et al., 2011).

The first step in creating a successful conservation biological control programme using flowering plants is the laboratory evaluation of candidate plants and their effects on the fitness of the pest(s) and natural enemies. This study examined the longevity (a proxy for fitness) of the fly and the parasitoid on different flowering plant cultivars (just fly was used for the cultivar evaluations) and species. The cultivar experiment examined cultivars taken from *Lobularia maritima* (L.) Desv. (sweet alyssum). In another experiment, *Phacelia tanacetifolia* 'Balo', Bentham, *Fagopyrum esculentum* Moench. cv. Katowase (buckwheat), and *Leptinella dioica*, Hook.f. (shore cotula), a New Zealand endemic species were used. The latter species was chosen based on its provision of a range of ecosystem services in horticulture (Shields et al., 2016) that included invertebrate conservation, pest development, enhancing predator densities and other variables important for plant growth. It is of note to report that *L. maritima* is a known host plant of *S. flava* but leaves are not made available to the insects (only the flowers) and they are not known to use the flowers' parts as a food source (only leaves and nectar). Buckwheat was suspected to have the greatest positive effect on longevity because of previous research (Nafziger & Fadamiro, 2011; Varennes et al., 2016). The overall goal was to find a flowering plant that benefited the parasitoid more than the pest.

4.2 Materials and Methods

S. flava and *A. sp. (nr. persimilis)* were kept in colonies in 60 x 60 x 60 cm BugDorms (<http://bugdorm.megaview.com.tw/>). *S. flava* was reared on 60cm x 30cm x 20cm trays of *Brassica juncea* 'Mizuna', which were kept at 22 ± 2°C in controlled temperature rooms with a 16 h day length. *A. sp. (nr. persimilis)* was introduced to a portion of the established *S. flava* colonies for rearing. Established colonies were defined as colonies that have had several generations of successful cohorts emerge from them. Cultivars of the two species were kept in separate rooms at

the Bio-Protection Research Centre (<https://bioprotection.org.nz>), Lincoln University, Lincoln, New Zealand. The *B. juncea* was made available to the flies when it was 3 weeks old. The flies and *A. sp. (nr. persimilis)* that were used for the flowering plant evaluations were females that were 2 ± 1 days old and were all chosen at random from their respective colonies.

4.2.1 Alyssum cultivar evaluations with *Scaptomyza flava*

The first experiment used different cultivars of alyssum. The method was adapted from Begum et al., (2004), who examined cultivars of alyssum with different flower colours. However, the six cultivars used in this experiment ('Bentharii White', 'New Carpet of Snow', 'Snowcloth', 'Tiny Tim', 'Compacta Minima', and 'Crystallina') all had white flowers. Only the flies were used in this group of experiments because there was a very limited number of seeds for some cultivars, as these were experimental and not publicly released. Single plants of each cultivar were presented at random with a single female *S. flava*. The flies were put into transparent plastic chambers (Fig. 4.1), each of which contained a single inflorescence as well as an Eppendorf tube filled with water and plugged with cotton wool, so the flies could maintain hydration. Controls consisted of a similar chamber, but contained no inflorescence. The chambers contained two foam plugs to allow access to the chamber. One was for the insertion of the inflorescence, while maintaining a seal that would not damage it and the second was a ventilation mesh. The fly numbers were monitored daily until all had died. Fly/cultivar combinations were replicated ten times and plants were only used once.

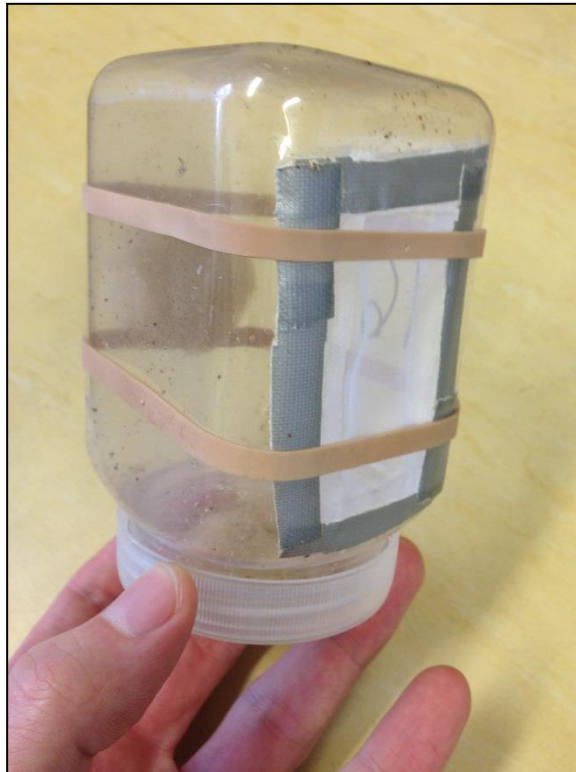


Figure 4.1 A chamber used to isolate a female *Scaptomyza flava* with an inflorescence. All containers had an Eppendorf tube filled with water and plugged with cotton for insect hydration.

4.2.2 Flowering plant species evaluations with *S. flava*

This experiment used cages with a single female *S. flava* with a single inflorescence spike of four test plant species and a water control. These were *L. maritima* 'Bentamii White', *P. tanacetifolia* 'Balo', *F. esculentum* 'Katowase', and *L. dioica*. The same chamber arrangement was used to monitor the flies until all had died. There were seven replicates because some plants died before experimentation began. Each of these plant species was chosen for the evaluations because of their frequent appearance in the biological control literature, except for *L. dioica* which is an endemic species and could be of particular use (see Section 1).

4.2.3 Flowering plant species evaluations with *A. sp. (nr. persimilis)*

The third and final experiment used a single female *A. sp. (nr. persimilis)* in each replicate as with the flies, except that *L. dioica* was removed because of its unreliable flowering under laboratory conditions. There were six replicates instead of seven because some plants died before the experiment was conducted (plants were not reused from the previous experiments).

4.2.4 Analysis

Tests

Survival was analysed in R studio (R version, 3.3.1) with the 'survival' (v.2.38, Heinze & Ploner, 2016) and 'coxphf' (v.1.12, Therneau & Grambsch, 2000) packages. This analysis was used because the information gathered comprised survival data by nature, so non-normal data of this type is thought to be better analysed in a less conventional way. However, the complex and perhaps equivocal nature of these results made it useful to run a simpler analysis as well. This was indeed done and the methods used and the rationale for selecting them are given below.

Rationale for survival tests

This approach was used in similar study systems (Lavandero *et al.*, 2005; Araj *et al.*, 2006). The initial analysis (survival) used Cox's proportional hazard model to test if survival time depended on the flower species used. Unfortunately, in this analysis some parameter estimates diverged to + or - infinity, a phenomenon known as monotone likelihood, which renders parameter estimates inaccurate (Heinze & Schemper, 2001). More specifically, "it has been recognized that under certain circumstances parameter estimates may diverge to infinity despite ... convergence of the likelihood," (Heinze & Dunkler 2008). In this work's context, this means that the treatments differed so greatly that it is difficult to compute 95% confidence intervals and, as a result, the lower CI and not the upper CI were often the only bounds reported, even though all treatments experienced "convergence of the likelihood" (death).

However, the data (median, means etc.) derived from the tests were still relevant and very probably significant (i.e. the model fit p-value was < 0.001). Because of problems caused by monotone likelihood, the procedure recommended by Heinze & Dunkler (2008) (a likelihood ratio test using Cox's proportional hazard model with Firth's correction) was used. This correction made the usage of the medians applicable because if the bounds were unreportable, but medians between treatments differed, they were highly likely to be different because their parameters diverged to infinity (i.e. treatments are very likely to be different from each other). This tested if the survival time in a given treatment (i.e. for a given species or alyssum cultivar, depending on the experiment) differed from that for the appropriate control group. In addition to the analysis of Cox's proportional hazard model, it is often useful to visualise how survival probability changes with time. In view of this consideration, Kaplan Meier curves were also plotted. When it was confirmed that there were probably differences among flower types, the flower that resulted in the highest median survival time was selected as the best.

Rationale for analysis of variance

For an analysis of variance, the key assumptions are that, as for any linear model, including ANOVA, constancy of variance and independence (the latter achieved by randomisation at the design stage). This is explained in Wood & Saville (2013). So, in this analysis, log transformation was used for both the fly and parasitoid data sets.

4.3 Results

4.3.1 Alyssum cultivar evaluations with *Scaptomyza flava* (survival analysis)

All treatments (i.e. alyssum cultivars) were significantly different ($P < 0.0001$) from the control, except 'Tiny Tim' ($P = 0.296$). The longevity of the insects (Table 4.1) did not significantly differ between the control, 'Tiny Tim', 'Crystallina', 'Compacta Minima' and 'Snow Cloth.' These conclusions were based on Cox's model (see Section 2.4). 'Benthamii White' and 'New Carpet of Snow' had higher survival rates than the rest but did not differ significantly from each other (Table 1). 'Benthamii White' was selected for use in the subsequent species evaluations because of its greatest positive impact on longevity (Table 4.1 and Fig. 4.2).

Table 4.1 A comparison between alyssum cultivars for *Scaptomyza flava* longevity. The upper confidence limits have not been reported because of monotone likelihood (see Section 4.2.4). The cultivar with the highest median survival time (hereafter the best) is denoted by an asterisk (*).

Cultivar	Median survival (days)	Lower confidence limits
Control	3	2
Crystallina	4	4
Benthamii White*	8	7
New Carpet of Snow	7	6
Compacta Minima	4	4
Snow Cloth	5	4
Tiny Tim	4	##

Monotone likelihood causes unbounded confidence limits.

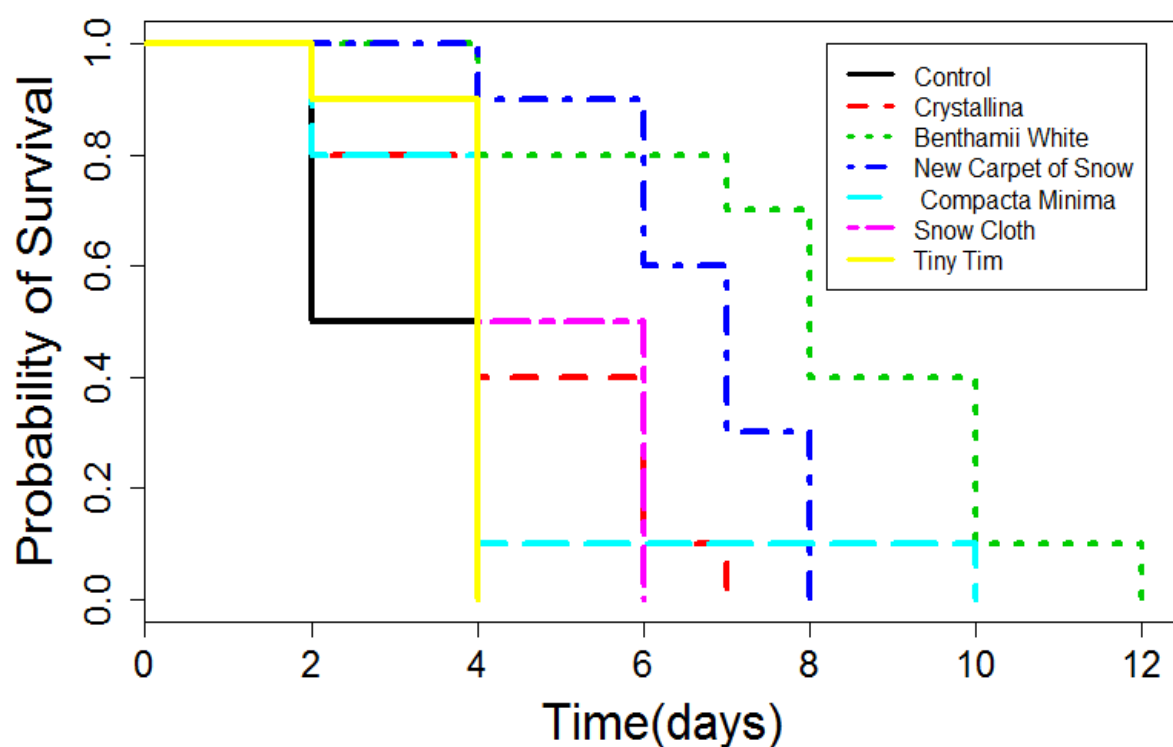


Figure 4.2 Kaplan-Meier survival curves of *Scaptomyza flava* adults on six flowering *Lobularia maritima* (alyssum) cultivars (see Table 1). The control line (black) reaches the x-axis at 4 days, as does that for ‘Tiny Tim’ (yellow line).

4.3.2 Flowering plant species evaluations with *S. flava* (survival analysis)

The survival analysis estimates show that treatment means were significantly different ($P < 0.01$) from the control, except *L. dioica* ($P = 0.234$). However, the longevity for all species overlapped, excluding alyssum (Table 4.2). This is apparent as the lower confidence limits (Table 4.2) overlapped with the control median. This indicates that *L. dioica*, *P. tanacetifolia* and *F. esculentum* are not significantly different from the water control, leaving alyssum (cv. ‘Benthamii White’) as the only treatment that differed significantly from the control.

Table 4.2 Longevity of *Scaptomyza flava* on five species of flowering plants. The upper confidence limits have not been reported because of monotone likelihood (see section 4.2.4). The species resulting in the highest median survival time (hereafter the ‘best’) is denoted with an asterisk (*).

Species	Common name	Median survival (days)	Lower confidence limits
Control	Control	4	2

<i>Leptinella dioica</i>	Leptinella	4	4
<i>Lobularia maritima</i>	Alyssum	6	6
<i>Phacelia tanacetifolia</i>	Phacelia	5	4
<i>Fagopyrum esculentum*</i>	Buckwheat	13	4

4.3.3 Flowering plant species evaluations with *A. sp. (nr. persimilis)* (survival analysis)

The survival analysis estimates show that treatment means are significantly different from the control ($P < 0.001$). However, analysis of the medians using the methods described in Section 2.2 indicate that alyssum is no better than the control (Table 4.3) and phacelia is no better than alyssum. This leaves buckwheat as the best candidate because it has the highest median survival (Figure 3.3).

Table 4.3 *Asobara sp. (nr. persimilis)* longevity on different flowering plant species. The upper confidence limits have not been reported because of monotone likelihood (see Section 4.2.4). the criterion to designate the best species was the same as Tables 1 and 2. The species with the highest median survival time is denoted with an asterisk (*).

Species	Common name	Median survival (days)	Lower confidence limits
Control	Control	5.5	4
<i>Lobularia maritima</i>	Alyssum	5	4
<i>Phacelia tanacetifolia</i>	Phacelia	6.5	5
<i>Fagopyrum esculentum*</i>	Buckwheat	7.5	7*

*Monotone likelihood causes inaccurate bounds

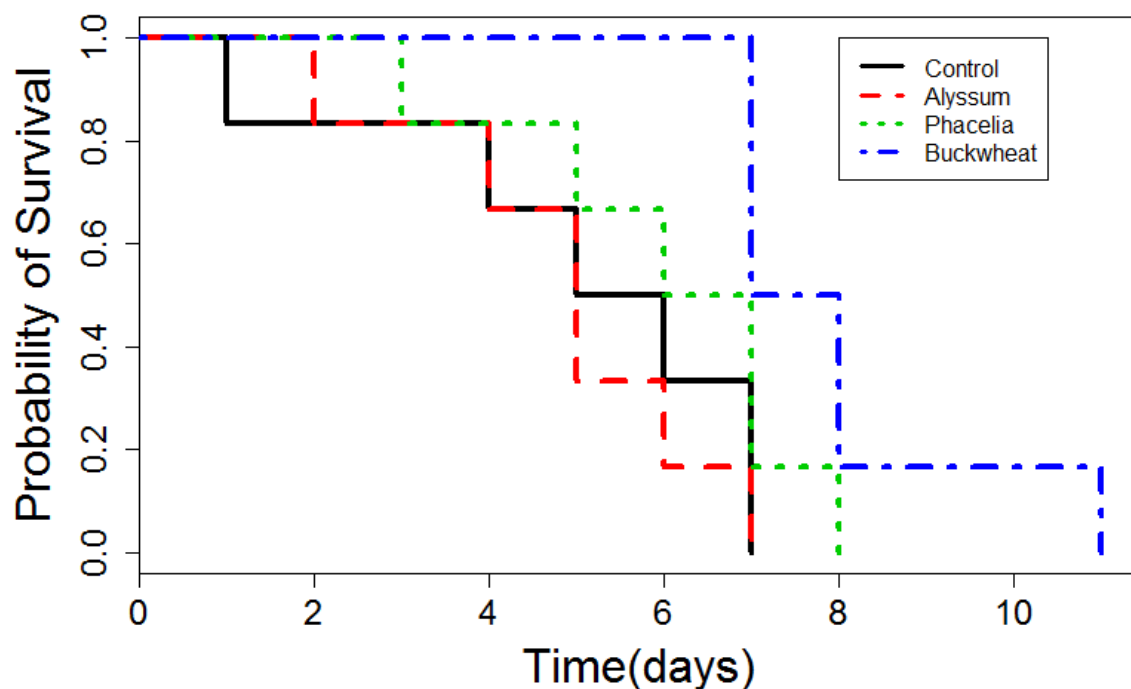


Figure 4.3 Kaplan-Meier survival curves of *Asobara sp. (nr. persimilis)* adults on 3 different flowering plant species (see Table 4.3).

4.3.4 Alyssum cultivar evaluations with *Scaptomyza flava* (ANOVA)

Scaptomyza flava survived the longest on ‘Benthamii White’ (approx. 8 days) and the shortest on just water (Table 4.4.) (approx. 3 days). ‘Benthamii White’ and ‘New Carpet of Snow’ were no different from each other but significantly different than all the other treatments ($P < 0.05$). ‘Snow Cloth’ and ‘Cystallina’ were significantly different than the control but none of the treatments below them (Table 4.4). The rest of the treatments (‘Compacta Minima’ and ‘Tiny Tim’) were no different than water.

Table 4.4 ANOVA table results of survival means for *Scaptomyza flava* on six different *L. maritima* cultivars and water controls. Data was non-parametric so a log transformation was used and then data in the second mean column was back-transformed for relatability.

Variety	Mean		Mean [#]	
BenthamiiWhite	0.884	a	7.65	a
NewCarpetofSnow	0.818	a	6.58	a
SnowCloth	0.660	b	4.57	b
Crystallina	0.619	b	4.16	b
CompactaMinima	0.582	bc	3.82	bc
TinyTim	0.572	bc	3.73	bc
Control	0.452	c	2.83	c
LSD(5%)	0.136		1.37	##LSRatio(5%)

[#]Back-transformed data

^{##}LSRatio – two means differ at $p < 0.5$ if their difference exceeds this number.

4.3.5 Flowering plant species evaluations with *S.flava* (ANOVA)

Scaptomyza flava survived longest on Buckwheat (approx. 10 days) and shortest on water (approx. 3 days) (Table 4.5). Buckwheat, alyssum and phacelia were no different than each other (Table 4.5). Buckwheat was significantly different than *Leptinella* and water (Table 4.5). Alyssum was significantly different than water (Table 4.5). Phacelia and *Leptinella* were no different than water (Table 4.5)

Table 4.5 ANOVA table results of survival means for *Scaptomyza flava* on four different plant species and water controls. Data was non-parametric so a log transformation was used and then data in the second mean column was back-transformed for relatability.

Species	Mean		Mean [#]	
Buckwheat	0.983	a	9.62	a
Alyssum	0.848	ab	7.05	ab
Phacelia	0.754	abc	5.67	abc
Leptinella	0.633	bc	4.29	bc
Control	0.501	c	3.17	c
LSD(5%)	0.263		1.83	##LSRatio(5%)

[#]Back-transformed data

^{##}LSRatio – two means differ at $p < 0.5$ if their difference exceeds this number.

4.3.6 Flowering plant species evaluations with *Asobara sp. (nr. persimilis)* (ANOVA)

Asobara sp. (nr. persimilis) survived longest on buckwheat (approx. 8 days) and shortest on water (approx. 4 days) (Table 4.6). Buckwheat significantly differ from all other treatments, while all other treatments were no better than water (Table 4.6).

Table 4.6 ANOVA table results of survival means for *Asobara sp. (nr. persimilis)* on three different plant species and water controls. Data was non-parametric so a log transformation was used and then data in the second mean column was back-transformed for relatability.

Species	Mean		Mean [#]	
Buckwheat	0.897	a	7.89	a
Phacelia	0.758	ab	5.73	ab
Alyssum	0.654	ab	4.51	ab
Control	0.628	b	4.25	b
LSD(5%)	0.266		1.85	##LSRatio(5%)

[#]Back-transformed data

^{##}LSRatio – two means differ at $p < 0.5$ if their difference exceeds this number.

4.4 Discussion

The results for the evaluation of alyssum cultivars with flies expand on a related study by Begum et al. (2004). However, the criterion for selecting the cultivars in that work was flower colour, which can influence insect behaviour as insects have been found to be attracted to different colors (Roubos & Liburd, 2009). The present study showed that there can be differences between cultivars on longevity even when the flowers appear to be the same colour. It is therefore important to research

different cultivars when comparing their effects on insects in the laboratory and field. For example, as in the present work, buckwheat is usually the most effective flowering plant species in conservation biological control and the cultivar Katowase dominates published work (Scarratt et al., 2008). Although in the context of this work, choosing buckwheat would seem counterintuitive, drosophilids and braconids have similar feeding habits for nectar as they both must push their way into the flower to attain nectar (Jervis *et al.*, 1993; Larson *et al.*, 2001). Attack rate *a sensu* Bailey et al. (1962) has been shown to be the most important variable in this respect and can improve when buckwheat nectar is provided to some parasitoids. As discussed below (Kean et al. 2003) have demonstrated the importance of search rate rather than fecundity in enhancing parasitoid 'fitness.' With this in mind, what is best for the fly's longevity may also be best for that of the wasp; but longevity is not the most important parameter in this case. Time and resourcing was also a factor so more cultivars were not chosen. In addition, more of these cultivars were not chosen because all were experimental except, Benthamii, as it was already available commercially.

The chemical signals from that plant to which parasitoids respond are beginning to be identified (Foti et al., 2015), but it remains largely unknown to what extent other buckwheat cultivars differ in the nature and strength of those signals and their effectiveness in enhancing biological control.

When evaluating different plant species and their effect on longevity, the outcome of the analyses supports the initial aim of this work, i.e., to find a flowering plant that benefitted the parasitoid but not the pest or at least, more than the pest. Although alyssum cv. 'Benthamii White' favoured *S. flava* longevity, it had no such significant effect on the parasitoid. For the latter, buckwheat was the only plant species that enhanced *Asobara* longevity. The experimental support here for the hypothesis that some flowering plant species can be selective in favour of the natural enemy could be criticised on the basis of replicate number. However, this work represented a technical challenge in not having all plant species available and in flower at the same time (see Section 2.2). Six or seven replicates were therefore used here but it should be noted that those numbers are similar to those in Berndt and Wratten (2005) who evaluated only one flowering plant species. The present study's findings also support other work that shows that flowering plants can be used to increase natural enemy 'fitness' and potentially enhance biological control as well (Scarratt et al., 2008; Tompkins et al., 2010; Gillespie et al., 2011). Here, all but *L. dioica* were species that had previous successes in enhancing biological control, but this study showed that most of the plant species evaluated (except buckwheat) were not significantly better than water. This suggests that there is a high probability that there may be an even better flowering plant species that was not used in this study. This is because flower morphology and nectar composition has been shown to affect fitness of natural enemies (Tylianakis et al., 2004; Wackers, 2004; Vattala et al., 2006; Cawoy et al., 2008; Winkler et al., 2009). Therefore, it would be useful to evaluate a wider range of plant families and species.

It follows that extensive screening for plant candidates may be necessary to successfully implement the best selective conservation biological control using flowering plants. However, the challenge of finding complete or partial selectivity, favouring the natural enemy, is likely to be greater in that case. Also, in screening for conservation biological control plant candidates, longevity is only one of the key natural enemy traits. Attack rates (Kean et al., 2003), proportion of time searching (Araj et al. 2011), other non-nectar resources (shelter, alternative food, pollen (Gurr et al., 2017), honeydew (Varennnes et al., 2014), hyperparasitism (Varennnes et al., 2014), larval or adult inter- and intra-specific competition (Poelman et al., 2013) and availability of alternative prey/hosts (Tylianakis et al., 2007) are important. Modelling and synthesis approaches can help in this regard (Perović et al., 2017) so that conservation biological control can be given a sound theoretical base. There is also an increasing emphasis in agroecology on multiple ecosystem services and these need to be considered in conservation biological control programmes (Shields et al., 2016).

Chapter 5

Creating the big picture: Abundance, distribution and movement of a leaf mining pest fly, *Scaptomyza flava* Fallén (Diptera: Drosophilidae) and its potential natural enemies in salad crops

5.1 Introduction

Flowering plants are commonly used to enhance conservation biological control in agroecosystems as many natural enemies feed on the nectar/pollen as resource subsidies from these plants (Scarratt et al., 2008; Orre Gordon et al., 2013; Balzan & Moonen, 2014). To determine if and to what effect the target insects are utilizing these floral resources, rubidium chloride can be mixed into a solution of water and sprayed on the plants (Scarratt et al., 2008). Rubidium follows the same pathway in plants as potassium so when sprayed at concentrations higher than background levels, marking of tissues can be detected (Corbett et al., 1996). Not only does the marking detect whether the plants are being utilized, but it can also empirically demonstrate how far an insect can travel after feeding on nectar. This is particularly important in agroecosystems to determine the pattern in which the flowering plants need to be sown, to create the most benefit to natural enemies (Scarratt et al., 2008).

Another way to examine the effects that flowering plants may have on the targeted insect community is to look at the distribution and abundance of the insects before and after the plants flower (Ramsden et al., 2014). Flowering plants have been shown to directly affect these in an insect community (Amaral et al., 2013; Ramsden et al., 2014). The distribution and abundance can then be mapped if GPS coordinates and insect presence are recorded at each sampling to give a snapshot of the differences before and after floral resources are present. These maps in combination with the rubidium chloride tracking can provide a richer picture of how the floral resources influence insect communities and how the agroecosystem should be manipulated to create the most beneficial layout of the flowers. This is to give the most benefit for both the insects and the growers. Most research to date focuses mainly on the natural enemies, as this gives a moderately good picture of how the floral resources potentially impact the agroecosystem. This is because it is assumed that if the fitness and/or numbers of natural enemies are enhanced then biocontrol is increased. However, this is not always the case as can be seen in cabbage fields where the overall parasitism incidence was not increased although longevity was (Lee & Heimpel, 2008). To avoid this pitfall, in the current work the pests were also examined to determine the relative benefit of flowers to them as well as the natural enemies.

This study used the approaches of Scarratt et al. (2008) and Corbett et al. (1996) as templates to design the experiments. *Scaptomyza flava* (Fallén) is the predominant pest in brassica crops in New Zealand (Seraj, 1994; Martin, 2012). Such plants, especially loose-leaf Asian brassicas, are used for the leaves, as these are consumed as pre-packaged salads. Little is known about the insect community in these agroecosystems in New Zealand besides the work conducted by Martin (2004, 2012) on related brassica crops. His work with *S. flava* primarily consisted of rearing them and their natural enemies from leaf material. This means that the natural enemy identities, life history of both insect groups and behaviour of both insect groups have been left for interpretation/discovery. This current work went further and investigated multiple parasitoid species to possibly tease apart their relationship with *S. flava*. The hypothesis was, can buckwheat change the abundance and distribution of *S. flava* and its parasitoids, this was based on prior work (Scarratt et al., 2008), the hypothesis includes the idea that buckwheat will change the distribution and abundance in favour of the fly's natural enemies (parasitoids).

5.2 Materials and Methods

The study site was an organic unit (BHU; <https://www.bhu.org.nz/>) with an 825m² plot that consisted of 11 *Brassica juncea* 'Mizuna' beds that ran approximately 50m in single rows. The BHU is on the Lincoln University campus, New Zealand. The sampling was conducted in the summer of 2015 and 2016 but, complications arose in 2015 and made it impossible to complete sampling and thus the data for 2015 were incomplete and not available for analysis. This left only 2016 data for analysis. *Fagopyrum esculentum* Monech 'Katowase', (buckwheat) was the flowering plant chosen, this was based on previous work (Scarratt et al. 2008). The buckwheat was sown on one side of the beds, running perpendicular to the crop beds. Each sampling point in the field was recorded with a GPS system (Garmin GPS60).

5.2.1 Field sampling

Sampling was performed before the buckwheat was sown to measure background rubidium levels in the insects. Six weeks after buckwheat was sown, Mizuna seed was planted so that the flowering of the buckwheat coincided with the commercial harvesting time of Mizuna (which is approximately 21 days). The initial sampling was completed using a Vortis suction sampler to collect insects for the analysis of background rubidium levels. The samples collected after buckwheat flowered were a combination of plant harvests (for mines and then rearing) and sticky traps. The traps were set up throughout the field (0, 0.5, 1, 2, 4, 8, 16 and 32m from the buckwheat strip) starting at the edge beds and moving every other bed until the entire field was covered (beds 1,3,5,7,9 and 11). The sticky traps were left for 7 days after the rubidium solution (1000ppm RbCl dissolved in 10L water) was sprayed on the flowering buckwheat. Before spraying, flowers were sampled for background

concentrations of rubidium. Then flowers were sampled every other day after spraying was completed to find the rubidium replacement curve in the flowers nectar reserves. Five flowers were chosen from each sampling period and put into individual 1.7ml Eppendorf tubes and these were taken from each odd numbered bed of buckwheat. A single flower sample was considered one inflorescence spike and the buckwheat samples were located relative to the Mizuna beds for ease of comparison.

5.2.2 Measuring rubidium

After the yellow sticky traps were collected, the insects were removed from the traps using turpentine and both targeted groups of insects (*S. flava* and parasitoids) were separated into their own individual 1.7ml Eppendorf tubes and labelled according to the bed they were sampled from and the distance from the buckwheat bed. *S. flava* and parasitoids were dried in an oven at 32-35°C for 24h and then weighed. This was done to adjust for weight, if weight was found to affect the rubidium concentrations (ppb) found in each insect. They were then submerged in 97% ethanol and frozen. To measure rubidium levels, the organisms needed to be digested. Before this digestion occurred, however, high quality photographs were taken of all the key characteristics of the parasitoids for retrospective identification, if rubidium concentrations were found to be significantly different from the control (before buckwheat was sown) and the information was considered interesting (e.g. equal number marked/unmarked or only few marked). After these photographs were taken, the insects were digested using a two-step tissue oxidation process outlined in Corbett et al. 1996. To oxidise (or digest) the tissues individual specimens were dried in an oven at 32-35°C for 24h to evaporate all ethanol and then 50µl of concentrated nitric acid was added and the digest was heated in an aluminium heat block at 53-55°C for 2h. After heating, 50µl of 30% hydrogen peroxide was added to it and heated at the same temperature for another 2h. After this period, the liquid was diluted with 1000 or 1500µl of deionised water and then analysed using an atomic absorption spectrophotometer (AAS), following the procedure in Scarratt et al. (2008). If the rubidium concentrations could not be read, they were then diluted with 500µl more, because if the concentrations were too high the AAS is inaccurate. The machine used was a GBC Scientific GF 3000 graphite furnace with a PAL 3000 Auto Sampler and a rubidium lamp at a wavelength of 780nm. Program parameters were 700°C for 20s charring and 2500°C for 1s of atomisation. The rubidium analyses of the insects were restricted to samples taken 0m and 0.5m from the buckwheat because of limited funding.

The flower buds were individually analysed as well. Five buds were chosen at random opposite the ends of 6 of the 11 Mizuna beds. This took place over a 7-day period with buds on alternate days being taken. In total, therefore, 30 buds were collect. Once these samples were collected they were

weighed and digested using the same procedure outlined above except they were always diluted to 2000 μ l because of the high concentrations of rubidium found in them.

5.2.3 Spatial abundance and distribution

ArcGIS v. 10.3 was the software used to run natural neighbour interpolation to generate abundance and distribution maps for *S. flava* and parasitoids (all parasitoids were grouped together because the identities were unknown and there were limited numbers). This was done to find the abundance and distribution before and after the buckwheat was planted.

5.2.4 Analysis

ANOVA was run in R studio (R v. 3.3.1) to discover if weight influenced rubidium readings (i.e. does a larger insect generally have more rubidium in it or vice versa). Insects were considered marked if they had rubidium concentrations at least 1 standard deviation above those of the pre-rubidium sprayed insects. This criterion was also used to determine marked buckwheat flowers (Scarratt et al., 2008).

5.3 Results

5.3.1 Background concentrations of rubidium

Weight had a significant effect on the concentrations of rubidium ($P < 0.0001$) so the weights were used to readjust the rubidium concentrations for each specimen. Marked flies were considered to be those with rubidium concentrations above 1545 parts per billion (ppb). This figure comprises the mean rubidium concentration for all *Scaptomyza* individuals analysed ($742.2 \pm 803.$). For parasitoids, the respective data were 1717 ppb (870.2 ± 846.6) and for the flower buds, the respective data were 20706 ppb (8462.8 ± 122243.3).

5.3.2 Marked specimens

25 of the 32 captured *Scaptomyza* were marked within 0.5m of the buckwheat, and 29 of 35 wasps were marked, 94 of the sampled 120 buckwheat flowers were marked after the rubidium was sprayed. 30% of the buckwheat flowers were marked two days after spraying, while 90% were marked after four days and all flowers were marked between 6 and 8 days. The location of the Mizuna bed did not significantly affect the rubidium concentrations ($P > 0.05$) of either the insects or the flowers.

5.3.3 Distribution and abundance maps

The distribution and abundance of the insects did change over time and can be seen when comparing the maps in Figure 5.2 (pre-and post-buckwheat effects on the flies respectively) and

Figure 5.3 (pre-and post-buckwheat effects on the wasps respectively). The highest fly density areas decreased by approximately 50% while the wasp parasitism rates increased by 4-fold in their highest parasitism regions. There is also an apparent attraction of both insect populations towards the buckwheat strip.

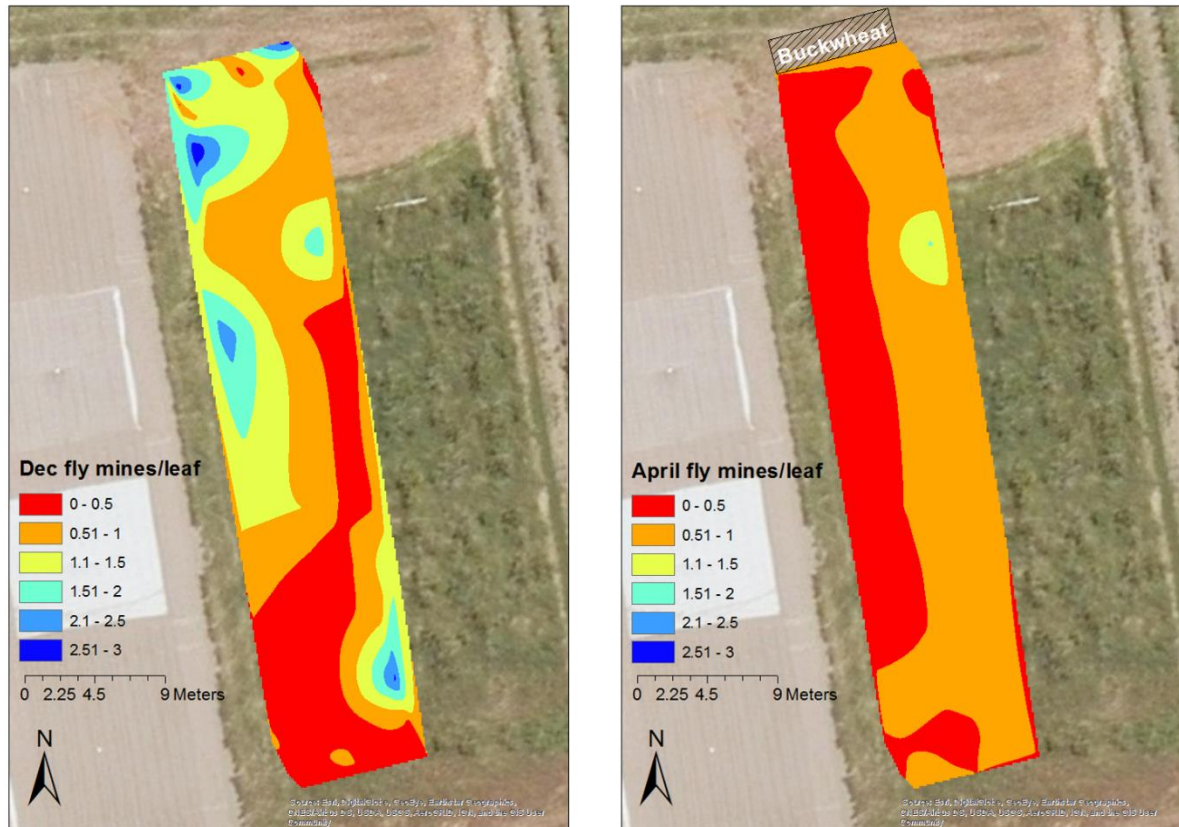


Figure 5.1 The maps display a ‘snapshot’ of the fly mines per leaf in late December 2015 (left) before the buckwheat was sown and the fly mines per leaf 6 weeks after buckwheat was sown, causing the sampling to occur in early April 2016 (right). The overall densities of the mines decrease with many of the “hotspots” in December completely evening out in April.



Figure 5.2 The maps display a ‘snapshot’ of the wasps reared out of leaf material in late December 2015 (left) before the buckwheat was sown and the wasps reared out of leaf material 6 weeks after buckwheat was sown, causing the sampling to occur in early April 2016 (right). There were generally fewer wasps reared in December while in April there was a “hotspot” near the highest densities of the flies, which happens to be close to the buckwheat.

5.4 Discussion

The results found here suggest that *S. flava* and most parasitoid wasp species found in this system feed on buckwheat and can be marked. Many articles have used rubidium to mark and record the consequences of insect movement since Scarratt’s (2008) work (Villegas et al., 2013; Klick et al., 2015; Madeira & Pons, 2015, 2016) but they tend to focus on just the beneficial insects. This study was conducted to find the behaviour consequences of the pest and natural enemies through observing their movement so that flowering plants (buckwheat) could be deployed more effectively. This study had the unexpected result of demonstrating buckwheat uptake and retention times for rubidium when the flowers are the targeted resource sink. Discovering these traits can help determine the protocols to access the movement and distribution of insects so that management of the pest can be targeted more effectively. This could include finding a better spatial pattern for the floral subsidies or to improve the extent to which the plant species deployed benefit the natural enemies more than the pests. Interestingly, the buckwheat did not express rubidium concentrations

immediately. This does make sense because the rubidium needs to travel up from the soil through the roots and ultimately into the flowers. It appears that with the soil conditions in this study it took approximately 6 days to attain 100% marking of flowers. This may suggest that false negatives could occur if sampling happened before the 6-day period and that a waiting period may need to occur (and be identified) before sampling in the field is conducted when using rubidium as a marker in flowering plants.

The rubidium results and the maps of the distribution and abundance suggest that buckwheat is a nectar source for a wide range of insects. In those, it appears to increase local parasitoid densities which could influence parasitism rates. Not only do the densities change before and after buckwheat is flowering/present but it also appears to alter the distribution by attracting both groups of insects closer to the flowering buckwheat. This supports the hypothesis that we can use flowering plants to alter the distribution and abundance of insects in the field and suggests this can also benefit the natural enemies. Many invertebrate marking techniques focus only on beneficial insects (Bowie et al., 1996; Dent 2000). This could be seen as being a little naïve as benefits of added nectar and pollen to non-target (in the context of this study) insects could confound the desired results of conservation biocontrol approaches.

Chapter 6

A survey of potential natural enemies of *Scaptomyza flava* obtained from yellow sticky traps related to distance from flowering buckwheat strip

6.1 Introduction

Apart from the detailed examination of potential biological control agents of *Scaptomyza flava* Fallén (chapter 5), it was considered that a broader assessment of potential natural enemies of the pest in the studied brassica crops would be useful. The guilds of natural enemies that may be of use in this context are likely to range widely from epigeal and climbing Coleoptera, spiders etc. to flying predators and parasitoids across a range of guilds and invertebrate families. This survey work was limited in space and time but the data generated could still be of use in future research on *S. flava*. The identities of potential biocontrol agents are essential because 1) specialists can be sought after for their lack of non-target effects (McEvoy, 1996; Marohasy, 1998; Van Lenteren *et al.*, 2006), and 2) the acronym ARMED can be deployed (see below). The aim of this chapter was to discover the potential natural enemies of the leaf mining fly *Scaptomyza flava* for use in developing a conservation biological control programme. This can facilitate the first steps in ARMED (Assess, Rank, Manipulate, Evaluate and Deploy; i.e. assess what potential natural enemies exist at the study site). This concept is developed further in Shields *et al.* 2018 and is referred to indirectly in Gurr *et al.*, 2017, Southwood & Henderson (2009) and Dent (2000).

The sampling carried out comprised only yellow sticky traps and the rearing of organisms emerging from *S. flava* mines. This was done at Lincoln and at an Auckland site; see Materials and methods section below. Presented here are tables and photographs which identify the main natural enemy types collected in this work. The organisms are identified as far as possible with limited taxonomic reference material available, but do give an overview of some of the representatives of the natural enemy guilds which could impact on *S. flava* eggs, larvae, pupae or adults.

6.2 Materials and methods

The Lincoln study site consisted of a 15m x 35m plot in an organic farm (Biological Husbandry Unit, BHU; <https://www.bhu.org.nz/>), on the Lincoln University campus. *Brassica juncea* (L.) Czern.

‘Mizuna’ was planted in 0.5m-wide beds the entire length (35m) of the plot on March 14th, 2016 while *Fagopyrum esculentum* Moench cv. Katowase (buckwheat) was planted 3 weeks before that on the northern end, perpendicular to the *B. juncea*. This was done to ensure that the buckwheat would be in flower during the invertebrate sampling period. Three weeks after sowing the *B. juncea*, yellow sticky traps were erected just above the canopy of the crops, starting at the edge beds and were erected every other bed (1,3,5,7,9,11) at the edge of the *F. esculentum* (0m) and then exponential distances away from this edge (0.5, 1, 2, 4, 8, 16, 32m). This made up 48 traps that were from Alpha Scents (<http://www.alphascents.com/>). They were 18 x 15cm and hung lengthwise (18cm vertical) from bamboo poles so that the bottom edge was 30cm from the plant tops centered in each bed (Yathorn et al. 1988). These traps were left for 7 days and then collected. The insects collected were then removed from the cards using turpentine and separated into individual Eppendorf tubes for identification.

The insects were stored in ~100% ethanol in a freezer. *Brassica juncea* ‘Mizuna’ leaves were also collected and put into perforated ‘bread bags’ with paper towelling. This was to keep humidity high so the leaves did not immediately desiccate and the paper collected any standing moisture. The bags were then left until leaves became brown and completely dehydrated (approx. 4 weeks). During this time, parasitoid wasps were removed from the bags as they emerged from the mines on the leaf material. Time limitations meant that work concentrated in detail only on insects easily identifiable to species. Other individuals were identified only to family. There were two groups of harvested material, material that came from the BHU and material that came from a commercial site in Auckland. The material from Auckland was a commercially harvested batch of highly infested material and was reared as stated above.

6.2.1 Keys

Most insects on the sticky traps were Hymenoptera, so a key published by Jacobson *et al.*, (1978) was used to determine species of *Vespula* (Vespidae). For the rest, the LUCID key, “What wasp is that?” was used to confirm initial family determinations (Stevens *et al.*, 2007). For the rest of the generalist natural enemies, they were easily identified to family using a general taxonomy text (Triplehorn & Johnson, 2005).

6.2.2 Analysis

Counts were compiled into three different tables. They were separated by: small Hymenoptera and generalists found on the sticky traps and reared material from the BHU and the Auckland site. These were then classified into insect family and distance from the *F. esculentum*, except at the Auckland site because the material collected was done so by commercial harvesters and distance was not recorded because they harvest entire beds at a time.

6.3 Results

6.3.1 Sticky traps (small Hymenoptera)

Ichneumonidae was the most prevalent family on the traps at 62% of hymenopteran individuals while Braconidae came second at 21% and Figitidae was third at 10% of the total number of small Hymenoptera found (Table 1). Approximately 90% of all Ichneumonidae captured were thought to be *Diadegma semiclausum* (Hellen) based on the Lincoln University PhD thesis of B. Lavandero (2004). 40% of individuals of all families had peak abundances at 1m and 2m from the buckwheat, constituting 80% of peak abundances for all families captured. There appeared to be two abundance peaks/family, one within 4m of the buckwheat and the other within 16m to the south edge (directly opposite of the buckwheat edge) that had no buckwheat. All the sticky trap material came from the BHU as traps could not be set up on commercial farms, because of normal farm actions including pesticide spraying.

6.3.2 Sticky traps (generalists)

Of all the generalists, Vespidae contributed to most individuals found (58%) with Hemerobiidae (brown lacewings) being second (27%) and Coccinellidae made up one third (12%) (Table 2). *Vespula*

vulgaris (L.) (*Vespidae*) made up 82% of all vespids found while *Vespula germanica* (F.) (*Vespidae*) made up the rest (18%), (Table 2). Hemerobiidae and Coccinellidae peak abundances were at 8m away from the buckwheat, while Nabidae and Vespidae peaks were between 2 and 4m from those plants (Table 2).

6.3.3 Reared Hymenoptera (BHU)

Eulophidae dominated the Hymenoptera reared from the leaf material with a 60% occurrence, Braconidae coming second at 20% and Diapriidae third with 12% (Table 3). Eulophidae and Ichneumonidae abundances appeared to peak at 4m from the buckwheat. Braconidae peaked at the same distances as the sticky-trap material (1-2m), while Figitidae and Diapriidae appeared to peak at 0.5m from the buckwheat.

6.3.4 Reared Hymenoptera (Auckland)

Torymidae generated most individuals reared from *B. juncea* 'Mizuna' material at 56% of all individuals. Next was Figitidae with 19% and lastly, tied at 13% were Eulophidae and Braconidae. Distances for this Auckland material were not recorded as it was taken from commercially harvested crops (stated above).

Table 6.1 Numbers of Hymenoptera captured on yellow sticky traps at exponential distances from a flowering strip of buckwheat (*Fagopyrum esculentum*). For position zero, traps were within the flowering buckwheat. The work was done at the Biological Husbandry Unit, Lincoln University, Lincoln, New Zealand and the traps were collected April 16th 2016.

Distance (m)	0	0.5	1	2	4	8	16	32	Total
<i>Braconidae</i>	2	7	23	27	20	22	33	11	145
<i>Diapriidae</i>	0	1	5	8	2	3	5	1	25
<i>Encyrtidae</i>	0	0	0	1	0	0	0	0	1
<i>Eulophidae</i>	1	0	0	0	0	0	0	0	1
<i>Figitidae</i>	0	0	11	8	4	8	13	27	71
<i>Formicidae</i>	0	0	0	0	1	0	0	0	1
<i>Ichneumonidae</i>	20	21	65	43	20	77	77	102	425
<i>Megaspilidae</i>	0	0	1	1	0	0	0	0	2
<i>Pteromalidae</i>	0	0	2	4	2	1	2	1	12
<i>Torymidae</i>	0	0	0	0	0	0	1	0	1
Total	23	29	107	92	49	111	131	142	684

Table 6.2 Counts of insect generalist natural enemies found on yellow sticky traps at exponential distances from *Fagopyrum esculentum*. The work was done at the Biological Husbandry Unit, Lincoln University, Lincoln, New Zealand and the traps were collected April 16th 2016.

<i>Distance (m)</i>	0	0.5	1	2	4	8	16	32	Total
<i>Coccinellidae</i>	1	1	0	3	3	8	1	1	18
<i>Hemerobiidae</i>	4	4	4	4	5	10	5	5	41
<i>Nabidae</i>	1	0	0	1	1	0	2	1	6
<i>Vespidae</i>	0	10	7	14	10	11	13	24	89

Table 6.3 Individual hymenopteran counts per family reared from *Brassica juncea* 'Mizuna' leaves taken from crop plants at the Biological Husbandry Unit, Lincoln University, Lincoln, New Zealand. See Materials and methods section and the traps were collected April 16th 2016.

<i>Distance (m)</i>	0	0.5	1	2	4	8	16	32	Total
<i>Braconidae</i>	0	0	1	1	0	1	2	0	5
<i>Diapriidae</i>	0	3	0	0	0	0	0	0	3
<i>Eulophidae</i>	1	0	0	0	11	1	1	1	15
<i>Figitidae</i>	0	1	0	0	0	0	0	0	1
<i>Ichneumonidae</i>	0	0	0	0	1	0	0	0	1

6.4 Discussion

A limited understanding of the species and functions of invertebrate natural enemies of pests is available in New Zealand. Most of this information relates to introduced or invasive pest species and their biological control agents (Valentine, 1975; Cumber *et al.*, 1977; Early, 1980; Macfarlane & Palma, 1987; Noyes, 1988; Donovan, 1989; Syrett, 1990; Harris, 1991; Barratt *et al.*, 1997; Sagarra *et al.*, 2001; Raman & Withers, 2003; Irvin *et al.*, 2006; Stevens *et al.*, 2007; Murray *et al.*, 2008) and new species are regularly being found e.g., Sivasubramaniam *et al.* (1997). This chapter represents a preliminary survey of natural enemy fauna associated with the main study crop in this thesis (*Brassica juncea*).

6.4.1 Sticky trap wasps

The Braconidae, Ichneumonidae and Figitidae dominated the trap catches and these families have been used in a range of biological control programs in New Zealand (Cumber *et al.*, 1977; Goldson *et al.*, 1992; Berry & Walker, 2004; Berndt *et al.*, 2006; Tomasetto *et al.*, 2016). The main parasitoid of *S.*

flava (*Asobara* sp. (nr. *persimilis*), Figure 6.1, Seraj, 1994) is in the Braconidae. Identifying individuals in this family to genus was impossible, although some of those captured were very similar to *A. sp.* (nr. *persimilis*) so some parasitism of *S. flava* by *Asobara* in this work is likely. This is also supported because a mass release of *A. sp.* (nr. *persimilis*) at the BHU was conducted in 2009 (MacDonald *et al.*, 2011). Diapriids were the fourth most abundant insect found in the sticky traps and are known to parasitize fly larvae (Early & Horning, 1978; Early, 1980; Stevens *et al.*, 2007) and were also found in the reared specimens (see below). This lends weight to the likelihood that some diapriids may be using *S. flava* as a host. There was one encyrtid found (Figure 6.2, Noyes, 1988), but this is unlikely to be of significance to *S. flava* as encyrtids are primarily parasitoids of scale insects (Stevens *et al.*, 2007). All figitids found on the yellow sticky traps were those that attack the brown lacewing (*Micromus tasmaniae* Walker (Hemerobiidae)) i.e., *Anacharis zealandica* Ashmead (Figitidae) (Irvin *et al.*, 2006) which can be seen in Figure 6.3. Identifying the ichneumonids was not necessary as most were too large to be of significance (Figure 6.4) to *S. flava* and the others were clearly *Diadegma semiclausum*, a key parasitoid of the diamondback moth (*Plutella xylostella*(L.)(Plutellidae)) (Figure 6.5, Lavandero *et al.*, 2005). The two megaspilids (Figure 6.6) could be parasitoids of almost any of the insects in this system. Although they are poorly studied in Australasia, they are known to parasitize lacewings, Diptera and scale insects (Hemiptera: Coccidae) (Stevens *et al.*, 2007), which means it is possible that *S. flava* could be an accidental or shifted host for one or more of them. The pteromalids (Figure 6.7) could be of interest because they parasitize leaf-miners and gall-inducers (Stevens *et al.*, 2007), but none was specifically reared from the leaf material, suggesting that these found on the sticky traps may not be associated with *S. flava*. Torymids are not known to parasitize drosophilids so the single torymid (Figure 6.8) from the sticky traps is probably of no interest (Syrett, 1990; Stevens *et al.*, 2007).

6.4.2 Generalist natural enemies on sticky traps

Brown lacewings (Figure 6.9), the German wasp (*Vespula germanica*, Figure 6.10), the common wasp (*Vespula vulgaris*, Figure 6.11), and the eleven-spotted ladybird beetle (*Coccinella undecimpunctata*, Figure 6.12) were the most abundant generalists caught on the sticky traps. Once *S. flava* had laid

their eggs, these insects could theoretically eat that life stage. Also, the vespids could prey on the adults (Harris 1991) or eggs of *Scaptomyza* but there is no published evidence for this the latter.

6.4.3 Material from BHU yellow traps

Five braconids species were reared from the leaf material. Some were clearly *Aphidius* spp. (Figure 6.13, Cameron & Walker, 1989), while the others are similar to *Asobara* spp. (Figure 6.1, Seraj, 1994). This may be related to the fact that *A. sp.* (nr. *persimilis*) was released in the area in 2009 (MacDonald *et al.*, 2011). The diapriids found were varied and comprise a probable *Trichopria* spp. (Figure 6.14, Grehan, 1990) and several *Spilomicrus* spp. (Figure 6.15, Early & Horning, 1978; Early, 1980) which are all parasitoids of flies (Stevens *et al.*, 2007). The eulophids (Figure 6.16) reared from the leaf material are interesting as this suggests they are either hyperparasitoids of whatever is parasitizing *S. flava* or parasitizing small eggs of another insect that was not detected on or from the material. Eulophids as a group have a large host range, but none is known primarily to parasitize drosophilids in New Zealand (Stevens *et al.*, 2007). The reared figitid is interesting because it is clearly not *A. zealandica* (Figure 6.17), although some figitids are known to parasitize small flies (Stevens *et al.*, 2007), which could mean this individual may have emerged from *S. flava* and not be an accidental contaminant, because screening of the material was done to remove all insects found that were larger than juvenile aphids. The ichneumonid on the other hand was probably from missed diamondback moth eggs and subsequent pupae.

6.4.4 Material reared from Auckland

The 'communities' of the Hymenoptera found in this material were very similar to the rearings from Lincoln material, apart from the torymids (Figure 6.18). This is an interesting group because they may be hyperparasitoids of already-existing parasitoids of either *S. flava* or any aphids that were present in the material. This is because as stated above the material was screened prior to the setup for rearing out the insects and again, Torymids are not known to parasitize *Drosophila* or aphids in New Zealand.

6.4.5 Final remarks

In summary, there may be some braconids, eulophids, figitids, and torymids of future interest in this region for biological control of brassica pests. Also, there may already be hyperparasitism occurring in these natural communities and thus could inhibit biological control of *Scaptomyza*. However, *A. sp.* (nr. *persimilis*) was chosen for this work because it is the only known parasitoid of *Scaptomyza* in New Zealand, has been successfully mass reared and access to insect colonies is readily available. As stated earlier, the survey work presented here is somewhat limited in time and location but gives an early indication of the potential guilds of natural enemies which could impact on *Scaptomyza* populations. As indicated at the beginning of this thesis, perhaps the biggest challenge in deploying conservation biological control for leaf-mining pests is that the insect's larval and pupal stages are likely to be unavailable to most natural enemy guilds, including some of the taxa identified in this survey. It is quite likely, however, that the fly's egg stage may be vulnerable to a range of guilds even though the eggs are laid within the leaf. The literature on this aspect of leaf-miner ecology is under-researched. However, for another genus of leaf-miner (*Stilbosis*) this was not the case (Faeth, 1985). The eggs of some leaf-miner species are indeed attacked by a suite of parasitoids but there is little or no literature on this dynamic for *S. flava*. The work of Seraj (1994: PhD thesis; Massey University) did identify some parasitoids in this guild but work on the dynamics of this interaction was not conducted.

Although yellow traps were used to catch members of these guilds, simulating floral resources, the role, if any, of flowers in the life history of many of the trapped insects is unknown. In fact, recent work on parasitoids and nectar point to the white flowers of buckwheat (*Fagopyrum esculentum* Moench.) being the most highly ranked rather than species with yellow inflorescences. Also, the work was not conducted in an agricultural monoculture but was surrounded by non-crop plants and habitat. That, and the discussion on the role of flowers above indicates that it is not possible to determine the extent to which the buckwheat flowers influence the spatial patterns observed. It is interesting that the only real pattern in the catches of these insects was that numbers were very low at position zero. At that site, the yellow trap was within the buckwheat plants, the biomass of which

was largely green leaf and stem material with the flowers around 0.5m above the trap. This suggests that the trap's yellow signal was swamped by the signal from the non-flowering parts of the plant.



Figure 6.1 A female *Asobara* sp. (nr. *persimilis*) adult taken from established laboratory colonies.



Figure 6.2 An encyrtid (sex unknown) caught on the yellow sticky traps at the BHU.



Figure 6.3 A figitid (sex unknown) found on yellow sticky traps at the BHU. All figitid specimens found on sticky traps were *Anacharis zealandica*, including this specimen.



Figure 6.4 A large ichneumonid (sex unknown) found on sticky traps at the BHU.



Figure 6.5 A *Diadegma semiclausum* (sex unknown) specimen found on yellow sticky traps at the BHU.



Figure 6.6 A megaspilid (sex unknown) found on yellow sticky traps at the BHU.

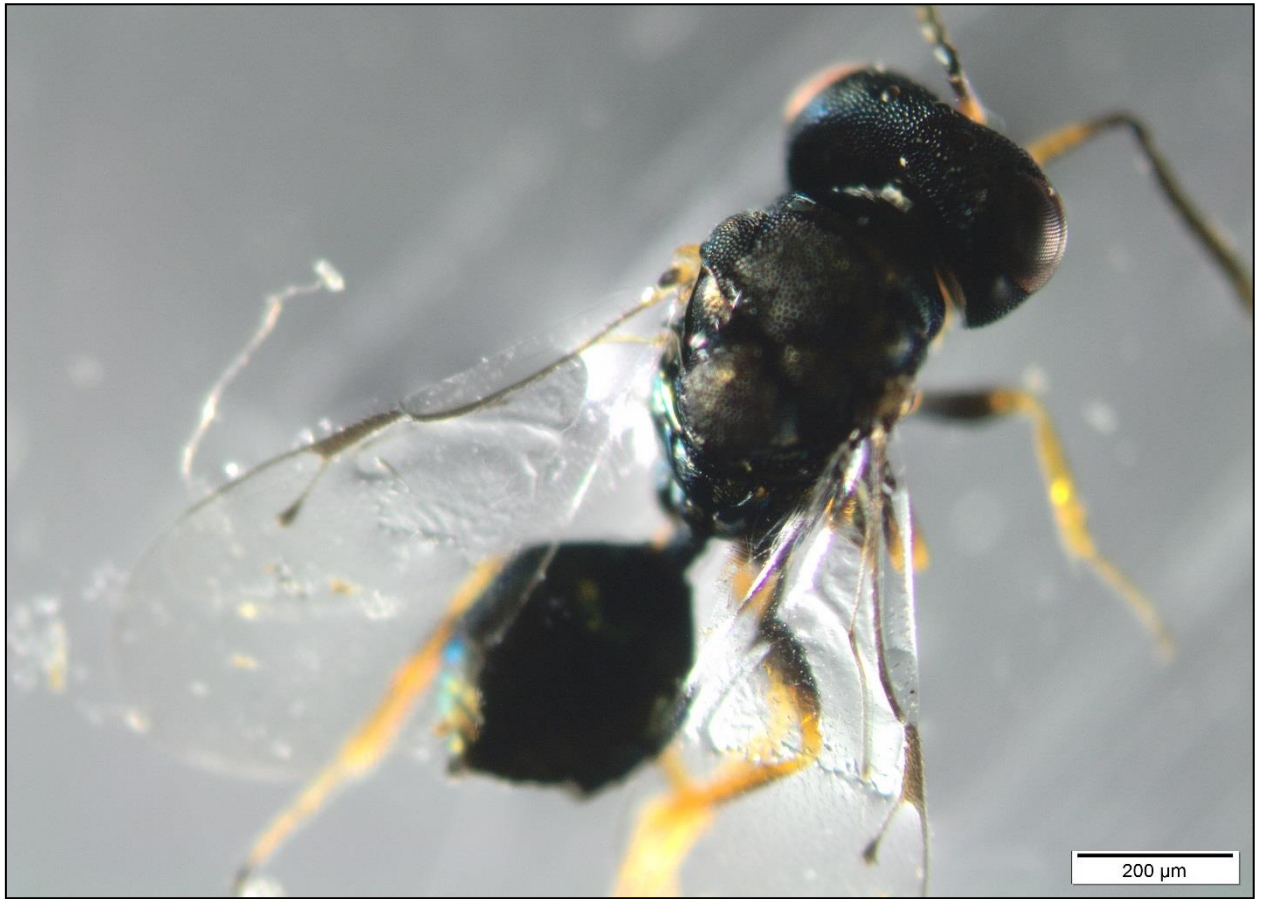


Figure 6.7 A pteromaidid (sex unknown) found on yellow sticky traps taken from the BHU.



Figure 6.8 The single torymid (sex unknown) found on the yellow sticky traps taken from the BHU.



Figure 6.9 A Brown lacewing (*Micromus tasmaniae*) found on yellow sticky traps taken from the BHU.

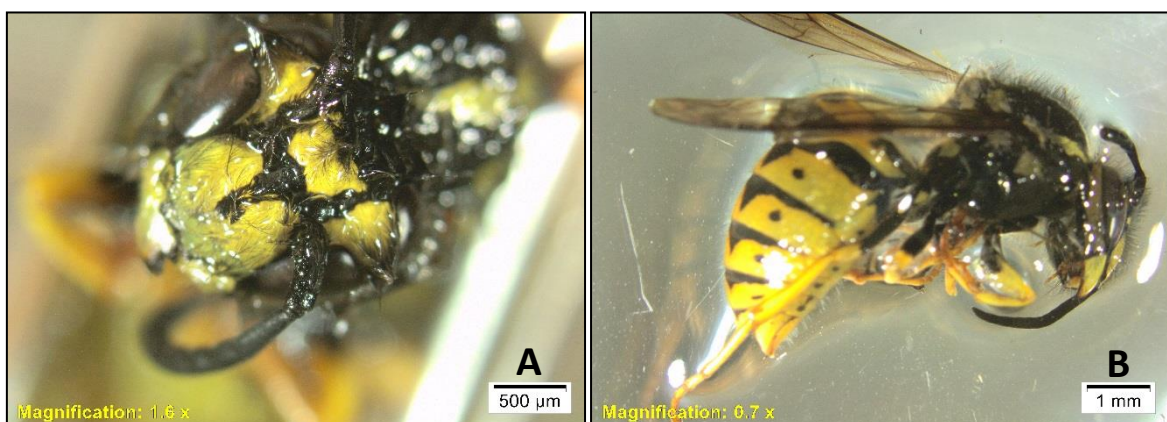


Figure 6.10 A *Vespula germanica* found on yellow sticky traps erected on the BHU. A – Face of *V. germanica* straight black line down centre of face is a distinguishing feature. B – Side view of *V. germanica* solid colour band behind eyes is a second identifying feature.



Figure 6.11 A *Vespa vulgaris* found on yellow sticky traps erected on the BHU. A – Face of *V. vulgaris* anchor shaped black mark down centre of face is a distinguishing feature. B – Side view of *V. vulgaris* band behind eyes interrupted by a black mark is a second identifying feature.



Figure 6.12 An eleven-spotted ladybird beetle (*Coccinella undecimpunctata*) found on yellow sticky traps taken from the BHU.



Figure 6.13 An *Aphidius* sp. (Braconidae) reared from plant material harvest from *Brassica juncea* 'Mizuna' grown on the BHU plot.



Figure 6.14 A *Trichopria* sp. (Diapriidae) reared from *Brassica juncea* 'Mizuna' leaves harvest from plants grown on the BHU plot.



Figure 6.15 A *Spilomicrus* sp. (Diapriidae) reared from *Brassica juncea* 'Mizuna' leaves harvest from plants grown on the BHU plot.



Figure 6.16 A representative of all eulophids reared from plant material harvested from *Brassica juncea* 'Mizuna' grown on the BHU plot.



Figure 6.17 A reared figitid from *Brassica juncea* 'Mizuna' material harvest on the BHU.



Figure 6.18 A reared torymid from *Brassica juncea* 'Mizuna' material harvest from the BHU.

Chapter 7

General Discussion

7.1 Main findings and their implications

The salad cropping and packaging business in New Zealand has a value of millions of dollars (\$NZ 53.7m/~\$US37.8m in 2015/2016) and should dictate that innovative ways to reduce costs while maintaining quality and quantity are highly sought after commodities (Wilkinson *et al.*, 2015). This PhD work partly aimed at finding such an innovative approach and found some prospects for reducing pesticide use. Conservation biological control is a now popular concept of pest control, although there are still many questions that need to be answered. It is also interesting to note that the group that needs the most guidance is the group that has had the least contact with the scientific body of knowledge (farmers) and it is scientists' social responsibility to disseminate this information in a way that reaches these groups, including developing a pathway to implementation (Warner, 2007).

At the start of this work, it was found that basic insect handling information was lacking so a comparison of anesthesia techniques was conducted. It was found that the common practices of insect handling may not have been the most appropriate, so another technique (the use of triethylamine) was ultimately the approach selected here. This decision concluded that the amount of time necessary to handle the insects was not feasible with the two common practices (chilling and CO₂), i.e., meeting a 15 min period for an anesthesia effect to occur, so the alternative was selected for use. It was also found that if handling many insects, the cost of using CO₂ became prohibitive, while using the alternative (triethylamine) was highly cost effective (~\$NZ 60 for four hours use vs. ~\$NZ 60 for multiple years' worth respectively). It is prudent to note that the sub-lethal effects of triethylamine were not exhaustively investigated here and would need additional work to make sure that other aspects of sub-lethal effects on the insects are not affected.

Having the new handling methodology (triethylamine) available created greater confidence in further experimentation. Triethylamine vapour was used to facilitate the selection of female insects and introduce them to flowering plants to determine if they affected their longevities. This is important because providing floral resources to increase populations and efficacy of beneficial insects is popular (Dainese *et al.*, 2017; Graves *et al.*, 2017; Mensah *et al.*, 2017, Gurr *et al.*, 2017), so it is important to know exactly what species and cultivar is being used. It is also important to note that buckwheat is a strong contender in most floral resource comparison studies as it frequently performs better than the others (Scarratt *et al.*, 2008; Foti *et al.*, 2017). This could be because buckwheat

originates across Eurasia (Ohnishi, 1998) and *S. flava* populations coexist within this range (Hackman, 1959) and as such have become adapted to using the nectar of buckwheat to survive periods lacking its host plants on which to oviposit. More likely is that this plant compounds which are particularly attractive to parasitoids and has a highly suitable floral morphology and nectar quality (Foti et. al., 2015).

Buckwheat laboratory results led to it being chosen as the plant to sow in the field. To attain a better understanding of the unmanipulated insect community at this site *Brassica juncea* 'Mizuna' was sown so that the harvesting period would coincide with the buckwheat flowering. Samples were taken of the buckwheat flowers and Mizuna (for insects) before a solution of rubidium chloride was applied to the flowers of buckwheat. This was to find the background rubidium levels.

The conclusions support those of other similar studies in that some flowering plants can be used to increase local populations of beneficial insects (Simpson *et al.*, 2011; Hoddle *et al.*, 2015; Dainese *et al.*, 2017; Mensah *et al.*, 2017; Zhao *et al.*, 2017) and rubidium chloride can be used to quantify insect movement to some extent (Scarratt *et al.*, 2008; Madeira & Pons, 2015, 2016). Other major conclusions here were that there is an anesthesia method that works better than the common practices. Also, alyssum cultivars differed in their effects on longevity for the pest species. This aspect of conservation biological control is rarely investigated (i.e. plant cultivars). For example, the buckwheat cultivar 'Katowase' (Irvin et. al., 2006) in conservation biological control dominates studies done in New Zealand. Buckwheat has many cultivars, but few have been investigated in this respect. Using alyssum has had the same potential limitations so that was addressed here. The cultivars were not evaluated against the studied parasitoid, however, because of sporadic availability of laboratory cultures outside of the summer season. In this context, maps were created that show a shift in distribution of the insects after buckwheat had flowered. All these together support the concept that flowering plants can be used to change the local distributions of insects and this may lead to pest population suppression. In fact, recent evidence suggests that even the colour yellow without any associated sugar reward can enhance oviposition rate by predatory hoverflies (Diptera: Syrphidae) in cereals (Day et. al., 2015). It may be the case that a white colour, as buckwheat flowers are, may induce a similar effect, but this remains contested. Of course, the hue, tint and intensity of 'yellow' or 'white' would need to be evaluated as well. Also, novel here was the first work on this global pest and its key parasitoid that showed that nectar can improve 'fitness.' Although the relative value of buckwheat to the longevity of *Scaptomyza* and *Asobara* did not differ between these two representatives of the second and third trophic levels, this does not necessarily exclude the possibility that the plant's nectar cannot improve *Asobara* fitness more than that of its fly host. This is discussed in chapter 4. Other parameters apply to a natural enemy's fitness and efficacy; such things as search rate and time spent searching are key (Kean et. al., 2003)

7.2 Conclusions

The work in this thesis addresses particularly challenging aspects of conservation biological control (Cons BC) in that the study system is one in which prophylactic use of pesticides is the norm (Daane et al., 2008d) and no economic threshold (ET) or economic injury levels (EIL) exist and much of the pest's cycle takes place within the leaf lamina. This does not necessarily exclude parasitoids (particularly specialist ones) but, many generalists' natural enemies would probably be excluded. Usually, when Cons BC is successful, pest populations are reduced and other ecosystem services are often aligned with that result (Jonsson *et al.* 2008). In those cases, an EIL has often already been established so the population below which pest damage can be tolerated is known. Hence, it is known if the Cons BC activities are enough to reduce pest populations below that level. Under a prophylactic regime, however, that simple calculation may not be appropriate because of the grower's wish to achieve zero pest damage. The company easily demonstrated, by brushing a hand through the growing crop, that large numbers of adult *S. flava* were active in the crop, so, in fact the intended prophylactic regime was not effective (Ashley Berrysmith, Snap Fresh Foods, personal communication). In the case of brassica salad crops, the focus of this study, that information means that a considerable amount of labour is still required in the packhouse to hand-sort for leaves damaged by *Scaptomyza* miners, even under this (flawed) pesticide regime. The deployment of appropriate flowering 'resource subsidies' for the BC agent, as used in this study could potentially reduce the proportion of damaged leaves entering the packhouse to lower levels especially, if the dependence on prophylaxis could be ameliorated. The flow on effects of this should include reduction of labour costs in the packhouse.

If the currently used prophylactic regime continued unchanged, then at least three things follow, despite the survival of many of the adult flies, indicating that such a regime was imperfect. It is possible that inefficient spray application technologies contributed to the persistence of the adult flies as well as continual immigration of the pest from weed hosts.

1. Many of the Cons BC agents are likely to be killed and a Cons BC regime will not be given the best opportunity to establish itself.
2. There will be no prospects for small reductions in the proportion of leaf damage, as the above regime is intended to result in no such damage.
3. Pesticide resistance may develop.
4. Another problem is that the rate of development of new agro-chemicals has been decreasing over the last 30 years (Williams and Kalmbach, 1943; Altbrod, 1977; Chen, 1987; Morita et al., 2007; Hardy et al., 2013; Spark and Naeen 2015, Cordova et al., 2016). This is

emphasized by Dr. Anne Thompson, Head of Development and Registration at DOW AgroSciences. Speaking at the “The Future of Weed Research” in London in 2008. To emphasize this challenge, Dr. Thompson said famously, “Please tell the farmers there is no cavalry coming over the hill.” Although this comment related to herbicides, the general situation for insecticides is likely the same.

Establishing a Cons BC regime that includes the addition of attractive non-crop plants to agriculture can however provide other ecosystem services (ES). These include having a positive impact on the wellbeing of the company’s employees, as well as on the marketing by companies which buy the produce and on the ultimate supermarket outlets. This ‘contentment’ ES has been clearly demonstrated to be effective in other, related work such as that of Forbes *et al.* (2009, which covered the ‘Greening Waipara’ project, <https://bioprotection.org.nz/greening-waipara>). With this in mind, the attempt to mitigate the effects of the usual monoculture may have an impact on the company’s attitude to spraying, the company may have to tolerate a higher rate of crop damage, albeit with the consequence of more time being necessary in the packhouse. However, the current work did not demonstrate a reduction in the number of leaf mines in relation to buckwheat deployment nor any impact of the extent to which pest number were reduced to below the ET or EIL. A completely different thesis plan would be necessary to address that. This was not done because the damage and ecology of the pest, and of the ecology of its natural enemies, was unknown at the start of this project.

Chilling	2	N/A	30	6	176	0
Chilling	2	N/A	60	20	464	0
Chilling	2	N/A	120	6	244	0
Chilling	2	N/A	240	6	270	0
Chilling	2	N/A	1080	6	915	0
Chilling	2	N/A	1440	6	570	0
Chilling	0	N/A	10	6	52	0
Chilling	0	N/A	30	6	128	0
Chilling	0	N/A	60	6	245	0
Chilling	0	N/A	120	6	511	0
Chilling	0	N/A	240	6	786	1
Chilling	0	N/A	1080	6	282	0
Chilling	0	N/A	1440	6	164	0
Chilling	4	N/A	10	6	50	0
Chilling	4	N/A	30	4	154	0
Chilling	4	N/A	60	8	35	0
Chilling	4	N/A	120	5	24	0
Chilling	4	N/A	240	3	48	0
Chilling	4	N/A	1080	4	12	0
Chilling	4	N/A	1440	4	10	0
TEA	N/A	50	80	N/A	N/A	20
TEA	N/A	100	65	N/A	N/A	20

* left overnight as they didn't recovery in an 8hr work day

** canister of gas would run out in approx. 5 h so couldn't do this trial as it was too long

Table A.2 Results from the Group 1 experiment. The estimates for the median recovery with the lower confidence limits and the mean deaths per replicate are given. The upper confidence limits are not reported because they were unbounded because of monotone likelihood. The anesthesia treatments chosen for Group 2 are highlighted in grey. The highest median recovery time and lowest death rate were used to determine the best treatments.

Within anesthesia technique (Group 1) effectiveness					
Application method	Intensity	Exposure	Median recovery (sec)	Lower 95% confidence limit of the mean (sec) [#]	Avg deaths per treatment after 24 h
CO ₂	N/A	5s	45	28.5	0
CO ₂	N/A	60s	102	83.5	0
CO ₂	N/A	600s	699	537	0
CO ₂	N/A	900s	1082	1081.5	0
CO ₂	N/A	1200s	1161	1161	0
CO ₂	N/A	1500s	1376	1376.5	0
CO ₂	N/A	1800s	4485	4383.2	3.333
Chilling	0°C	2h	242.5	18	0
Chilling	0°C	4h	262	435.5	0.333
Chilling	0°C	8h	268	95	0

Chilling	0°C	16h	304	215	0
Chilling	0°C	24h	606	188.5	1
Chilling	2°C	2h	47.5	0	0
Chilling	2°C	4h	17	0	0
Chilling	2°C	8h	54.5	12	0
Chilling	2°C	16h	73	0	0
Chilling	2°C	24h	50.5	30.5	0
Chilling	4°C	2h	33	6	0
Chilling	4°C	4h	26	14.5	0
Chilling	4°C	8h	12.5	3.5	0
Chilling	4°C	16h	13.5	3	0
Chilling	4°C	24h	11	6.5	0.333
TEA	25%	10s	3600	60	1
TEA	25%	20s	3600	N/A	0
TEA	25%	30s	6492	93	0
TEA	25%	40s	5568	72	0
TEA	25%	50s	5514	84	0
TEA	25%	60s	5670	64	0
TEA	50%	10s	6240	87	0
TEA	50%	20s	7080	100	0
TEA	50%	30s	6720	95	0
TEA	50%	40s	7200	93	0
TEA	50%	50s	7920	93.8	0
TEA	50%	60s	8760	138	0
TEA	75%	10s	5490	66	0
TEA	75%	20s	7020	60	1
TEA	75%	30s	6570	95	0
TEA	75%	40s	6840	113	0
TEA	75%	50s	12240	147	1
TEA	75%	60s	11052	157	0
TEA	100%	10s	8520	133	1
TEA	100%	20s	10680	171	0
TEA	100%	30s	12120	186	0
TEA	100%	40s	11160	159	1
TEA	100%	50s	12000	156	1
TEA	100%	60s	14220	195	1

#Upper confidence limits could not be estimated so they are not reported, see text for a more detailed explanation.

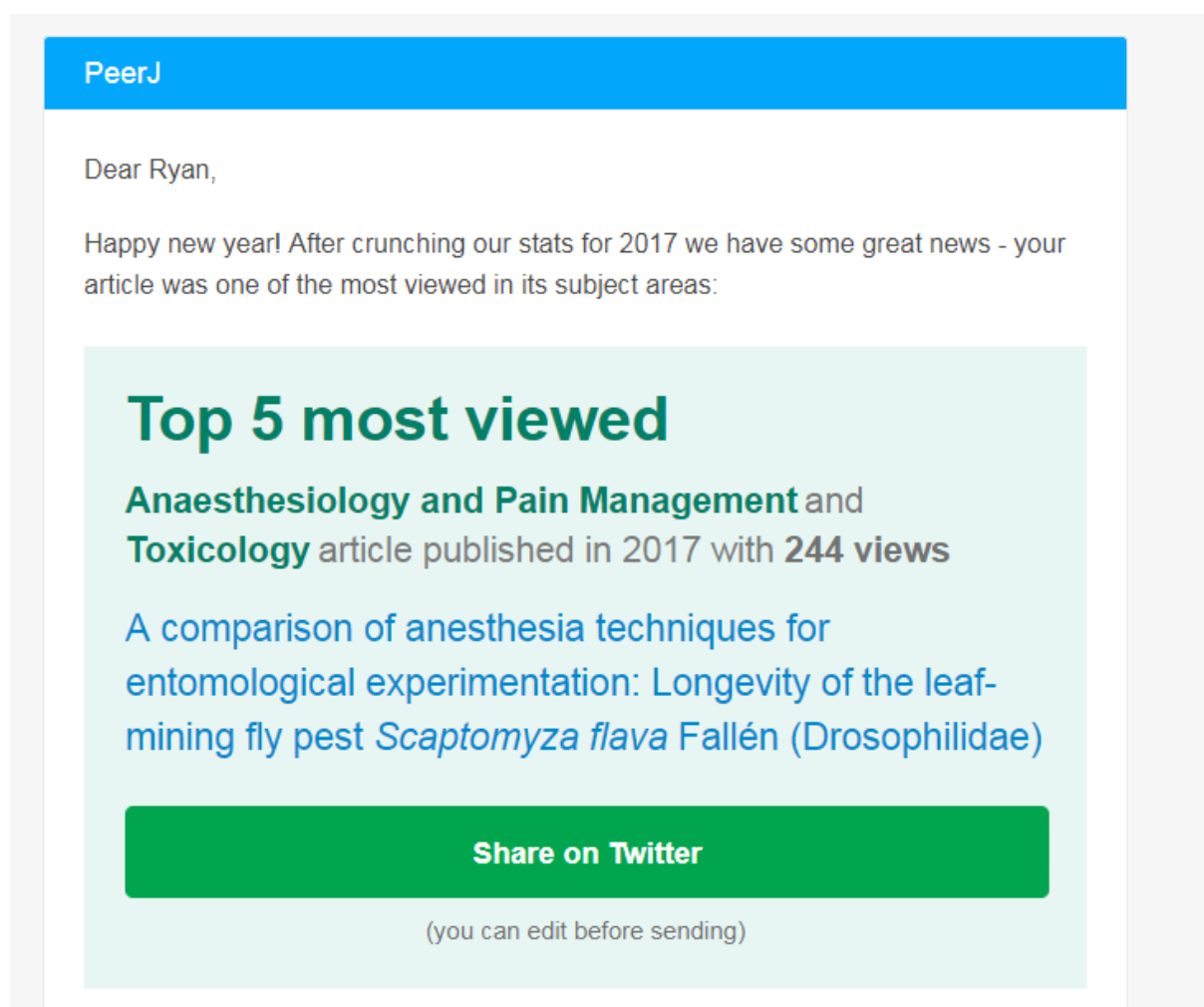


Figure A.3 Evidence that anesthesia information is sought after. This is a preprint and not a peer-reviewed article. This is important because even with this informal format, there is a high interest in it, lending weight that the publication of this material is of interest.

References

- Abhilash, P.C. & Singh, N. (2009) Pesticide use and application: an Indian scenario. *Journal of Hazardous Materials*, 165: 1–12.
- Aker, J.C. (2011) Dial “A” for agriculture: a review of information and communication technologies for agricultural extension in developing countries. *Agricultural Economics*, **42**(6): 631–647.
- Allard, V., Ourcival, J.M., Rambal, S., Joffre, R. & Rocheteau, A. (2008) Seasonal and annual variation of carbon exchange in an evergreen Mediterranean forest in southern France. *Global Change Biology*, 14: 714–725.

- Altbrod, J. (1977) Modern soil disinfection and weed control under glass. *Acta Horticulturae*, 58.
- Altieri, M.A. (1999) The ecological role of biodiversity in agroecosystems. *Agriculture, Ecosystems and Environment*, 74: 19–31.
- Amaral, D.S.S.L., Venzon, M., Duarte, M.V. a, Sousa, F.F., Pallini, A. & Harwood, J.D. (2013) Non-crop vegetation associated with chili pepper agroecosystems promote the abundance and survival of aphid predators. *Biological Control*, 64: 338–346.
- Amorós-Jiménez, R., Pineda, A., Fereres, A. & Marcos-García, M.Á. (2014) Feeding preferences of the aphidophagous hoverfly *Sphaerophoria rueppellii* affect the performance of its offspring. *BioControl*, 59: 427–435.
- Antiqueira, P.A.P. & Romero, G.Q. (2016) Floral asymmetry and predation risk modify pollinator behavior, but only predation risk decreases plant fitness. *Oecologia*, **181**: 475–485. DOI: 10.1007/s00442-016-3564-y
- Araj, S., Wratten, S., Lister, A. & Buckley, H. (2006) Floral nectar affects longevity of the aphid parasitoid *Aphidius ervi* and its hyperparasitoid *Dendrocercus aphidum*. *New Zealand Plant Protection*, 59: 178–183.
- Araj, S.-E., Wratten, S., Lister, A. & Buckley, H. (2008) Floral diversity, parasitoids and hyperparasitoids – A laboratory approach. *Basic and Applied Ecology*, 9: 588–597.
- Araj, S-E., Wratten, S.D., Lister, A., Buckley, H., & Ghabeish, I. (2011) Searching behaviour of an aphid parasitoid and its hyperparasitoid with and without floral nectar. *Biological Control*, 57: 79–84.
- Araujo, T.A. de, Picanco, M.C., Ferreira, D. de O., Campos, J.N.D., Arcanjo, L. de P. & Silva, G.A. (2017) Toxicity and residual effects of insecticides on *Ascia monuste* and predator *Solenopsis saevissima*. *Pest Management Science*: 1–26.
- Artiss, T. & Hughes, B. (2007) Taking the headaches out of anesthetizing *Drosophila*. *The American Biology Teacher*, 69: e77–e80.
- Badre, N. H., M. E. Martin, & Cooper, R.L. (2005) The physiological and behavioral effects of carbon dioxide on *Drosophila melanogaster* larvae. *Comparative Biochemistry and Physiology, Part A, Molecular & Integrative Physiology*, 140: 363–376.
- Bailey, V.A., Nicholson, A.J. & Williams, E.J. (1962) Interaction between hosts and parasites when some host individuals are more difficult to find than others. *Journal of Theoretical Biology*, **3**: 1–18.

Barratt, B.I.P., Evans, A.A., Ferguson, C.M., Barker, G.M., McNeill, M.R. & Phillips, C.B. (1997)

Laboratory nontarget host range of the introduced parasitoids *Microctonus aethiopoides* and *M. hyperodae* (Hymenoptera: Braconidae) compared with field parasitism in New Zealand.

Environmental Entomology, 26: 694–702.

Balkenhol, N. & Landguth, E.L. (2011) Simulation modelling in landscape genetics: On the need to go further. *Molecular Ecology*, 20: 667–670.

Balmer, O., Géneau, C.E., Belz, E., Weishaupt, B., *et al.* (2014) Wildflower companion plants increase pest parasitation and yield in cabbage fields: experimental demonstration and call for caution.

Biological Control, 76: 19–27. DOI: 10.1016/j.biocontrol.2014.04.008

Balzan, M. V. & Moonen, A.-C. (2014) Field margin vegetation enhances biological control and crop damage suppression from multiple pests in organic tomato fields. *Entomologia Experimentalis et Applicata*, 150: 45–65.

Barbosa, P. & Wratten, S.D. (1996) Influence of plants on invertebrate predators: Implications to conservation biological control. In *Conservation Biological Control* (ed. by DeBach, P.). Chapman and Hall, London, pp. 83–100.

Barnes, A.M., Wratten, S.D. & Sandhu, H.S. (2009) Harnessing biodiversity to improve vineyard sustainability. *Outlooks on Pest Management*, 20(6): 250–255. DOI: 10.1564/20dec04

Barron, A. B. (2000) Anaesthetising *Drosophila* for behavioural studies. *Journal of Insect Physiology*, 46: 439–442.

Batary, P., Baldi, A., Kleijn, D. & Tschardt, T. (2011) Landscape-moderated biodiversity effects of agri-environmental management: a meta-analysis. *Proceedings of the Royal Society B: Biological Sciences*, 278: 1894–1902.

Begg, G.S., Cook, S.M., Dye, R., Ferrante, M., *et al.* (2017) A functional overview of conservation biological control. *Crop Protection*, 97: 145–158. DOI: 10.1016/j.cropro.2016.11.008

Begum, M., Gurr, G.M., Wratten, S.D. & Nicol, H.I. (2004) Flower color affects tri-trophic-level biocontrol interactions. *Biological Control*, 30: 584–590.

Begum, M., Gurr, G.M., Wratten, S.D., Hedberg, P.R. & Nicol, H.I. (2006) Using selective food plants to maximize biological control of vineyard pests. *Journal of Applied Ecology*, 43: 547–554. DOI:

10.1111/j.1365-2664.2006.01168.x

Bennett, E.M. & Chaplin-Kramer, R. (2016) Science for the sustainable use of ecosystem services. *Biological Conservation*, 204: 449-458.

Bennett, N.J. & Dearden, P. (2014) Why local people do not support conservation: Community perceptions of marine protected area livelihood impacts, governance and management in Thailand. *Marine Policy*, 44: 107–116.

Berndt, L.A. & Wratten, S.D. (2005) Effects of alyssum flowers on the longevity, fecundity and sex ratio of the leafroller parasitoid, *Dolichogenidea tasmanica*. *Biological Control*, 32: 65–69.

Berndt, L. A., Wratten, S. D. & Scarratt, S.L. (2006) The influence of floral resource subsidies on parasitism rates of leafrollers (Lepidoptera : Tortricidae) in New Zealand vineyards. *Biological Control*, 37: 50–55.

Berry, J.A. & Walker, G.P. (2004) *Meteorus pulchricornis* (Wesmael) (Hymenoptera: Braconidae: Euphorinae): An exotic polyphagous parasitoid in New Zealand. *New Zealand Journal of Zoology*, **31**: 33–44.

Bianchi, F.J.J. a, Booij, C.J.H. & Tscharntke, T. (2006) Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. *Proceedings. Biological sciences / The Royal Society*, 273: 1715–1727.

Binkley, S. (2016) Fruit flies for the classroom. *Carolina.com*, 1–2.

Birch, A.N.E. & Begg, G.S. (2010) The future central role of IPM in EU crop protection: How can ecological research be put into practice? *Proceedings Crop Protection in Northern Britain*: 9–14.

Blommers, L.H.M. (1994) Integrated Pest Management in European Apple Orchards. *Annual Review of Entomology*, 39: 213–241.

Boggs, C.L. (2016) The fingerprints of global climate change on insect populations. *Current Opinion in Insect Science*, 17: 69–73.

Bompard, A., Jaworski, C.C., Bearez, P. & Desneux, N. (2013) Sharing a predator: Can an invasive alien pest affect the predation on a local pest? *Population Ecology*, 55: 433–440.

Boppana, S. & Hillyer, J.F. (2014) Hemolymph circulation in insect sensory appendages : functional mechanics of antennal accessory pulsatile organs (auxiliary hearts) in the mosquito *Anopheles gambiae*. *Journal of Experimental Biology*, 217: 3006–3014.

Bottrell, D.G. & Smith, R.F. (1982) Integrated pest management control has dim prospects for increased federal support. *Environmental Science & Technology*, 16: 282–288.

Bourguet, D. & Guillemaud, T. (2016) The hidden and external costs of pesticide use. *Sustainable Agriculture Reviews*. Springer International Publishing, Switzerland.

Bowie, M.H., Wratten, S.D., Evans, A.M. (1996) Movement of the hoverfly *Melanostoma fasciatum* in cultivated land. Proceedings of the 45th Annual Conference of the Entomological Society of New Zealand, p 54.

Brimner, T.A. & Boland, G.J. (2003) A review of the non-target effects of fungi used to biologically control plant diseases. *Agriculture, Ecosystems and Environment*, 100: 3–16. DOI: 10.1016/S0167-8809(03)00200-7

Buchanan, A.L., Hermann, S.L., Lund, M. & Szendrei, Z. (2017) A meta-analysis of non-consumptive predator effects in arthropods: the influence of organismal and environmental characteristics. *Oikos*, 126(9): 1233–1240. DOI: [10.1111/oik.04384](https://doi.org/10.1111/oik.04384)

Cameron, P.J. & Walker, G.P. (1989) Release and establishment of *Aphidius* spp (Hymenoptera: Aphidiidae), parasitoids of pea aphid and blue green aphid in New Zealand. *New Zealand Journal of Agricultural Research*, 32: 281–290.

Cannon, R.J.C. (1998) The implications of predicted climate change for insect pests in the UK, with emphasis on non-indigenous species. *Global Change Biology*, 4, 785–796.

Carvalho, F.P. (2006) Agriculture, pesticides, food security and food safety. *Environmental Science & Policy*, 9: 685–692.

Cawoy, V., Kinet, J., Jacquemart, A. (2008) Morphology of nectaries and biology of nectar production in the distylous species *Fagopyrum esculentum*. *Annals of Botany*, 102: 675–684.

Chacoff, N.P. & Aizen, M.A. (2006) Edge effects on flower-visiting insects in grapefruit plantations bordering premontane subtropical forest. *Journal of Applied Ecology*, 43: 18–27.

Champion De Crespigny, F. E. & Wedell, N. (2008) The impact of anaesthetic technique on survival and fertility in *Drosophila*. *Physiological Entomology*, 33: 310–315.

- Chan, P. (1987). *Bonsai Masterclass*. Boston: Sterling Publishing Company, Inc.
- Chaplin-Kramer, R., Valpine, P. de, Mills, N.J. & Kremen, C. (2013) Detecting pest control services across spatial and temporal scales. *Agriculture, Ecosystems and Environment*, 181: 206–212.
- Chapman, J.W., Drake, V.A. & Reynolds, D.R. (2011) Recent insights from radar studies of insect flight. *Annual Review of Entomology*, 56: 337–356.
- Chen, F., Ge, F. & Parajulee, M.N. (2005) Impact of elevated CO₂ on tri- trophic interactions of *Gossypium hirsutum*, *Aphis gossypii*, and *Leis axyridis*. *Environmental Entomology*, 34: 37–46.
- Chen, W. & Hillyer, J.F. (2013) FlyNap (Triethylamine) Increases the heart rate of mosquitoes and eliminates the cardioacceleratory effect of the neuropeptide CCAP. *PLoS ONE*, 8: 1–12.
- Chen, X., Rohrig, E. & Stansly, P.A. (2013) Carbon dioxide anesthesia of *Tamarixia radiata* (Hymenoptera: Eulophidae) parasitoid of *Diaphorina citri* (Hemiptera: Psyllidae). *Florida Entomologist*, 96: 246–248.
- Cheng, Y.C. & Lin, C.P. (2016) Dietary niche partitioning of *Euphaea formosa* and *Matrona cyanoptera* (Odonata: Zygoptera) on the basis of DNA barcoding of larval feces. *Journal of Insect Science*, 16: 73.
- Chisholm, P.J., Gardiner, M.M., Moon, E.G. & Crowder, D.W. (2014) Tools and techniques for investigating impacts of habitat complexity on biological control. *Biological Control*, 75: 48–57.
- Clough, Y., Barkmann, J., Juhbandt, J., Kessler, M., Wanger, T.C., Anshary, A., Buchori, D., Cicuzza, D., Darras, K., Putra, D., D., Erasmi, S., Pitopang, R., Schmidt, C., Schulze, C.H., Seidel, D., Steffan-Dewenter, I., Stenchly, K., Vidal, S., Weist, M., Wielgoss, A.C. & Tschardtke, T. (2011) Combining high biodiversity with high yields in tropical agroforests. *Proceedings of the National Academy of Sciences*, 108: 8311–8316.
- Colinet, H., V. Larvor, M. Laparie, M. & Renault, D. (2012) Exploring the plastic response to cold acclimation through metabolomics. *Functional Ecology*, 26: 711–722.
- Colinet, H. & Renault D. (2012) Metabolic effects of CO₂ anaesthesia in *Drosophila melanogaster*. *Biology Letters*, 8(6): 1050-1054.
- Cook, S.M., Khan, Z.R. & Pickett, J. A. (2007) The use of push-pull strategies in integrated pest management. *Annual Review of Entomology*, 52: 375–400.
- Cooper, J. E. (2011) Anesthesia, analgesia, and euthanasia of invertebrates. *Institute for Laboratory Animals Research Journal*, 52: 196–204.

- Cooper, J. & Dobson, H. (2007) The benefits of pesticides to mankind and the environment. *Crop Protection*, 26: 1337–1348.
- Corbett, A., Murphy, B.C., Rosenheim, J.A. & Bruins, P. (1996) Labeling an egg parasitoid, *Anagrus epos* (Hymenoptera: Mymaridae), with rubidium within an overwintering refuge. *Environmental entomology*, 25: 29–38.
- Costanza, R., Wilson, A., Troy, A., Voinov, A. & Liu, S. (2006) *The Value of New Jersey's Ecosystem Services and Natural Capital*. New Jersey Department of Environmental Protection. Burlington.
- Costanza, R., Kubiszewski, I., Giovannini, E., Lovins, H., *et al.* (2014) Development: time to leave GDP behind. *Nature*, 505: 283–285. DOI: 10.1038/505283a
- Costanza, R., de Groot, R., Braat, L., Kubiszewski, I., *et al.* (2017) Twenty years of ecosystem services: how far have we come and how far do we still need to go? *Ecosystem Services*, 28: 1–16. DOI: 10.1016/j.ecoser.2017.09.008
- Cooper, J. & Dobson, H. (2007) The benefits of pesticides to mankind and the environment. *Crop Protection*, 26: 1337–1348. DOI: 10.1016/j.cropro.2007.03.022
- Crowder, D.W. & Jabbour, R. (2014) Relationships between biodiversity and biological control in agroecosystems: Current status and future challenges. *Biological Control*, 75: 8–17.
- Cullen, R., Warner, K.D., Jonsson, M. & Wratten, S.D. (2008) Economics and adoption of conservation biological control. *Biological Control*, 45: 272–280. DOI: 10.1016/j.biocontrol.2008.01.016
- Cumber, R.A., Allan, D.J. & Helmore, L. (1977) Introduction and successful establishment in New Zealand of further strains of *Apanteles ruficrus* Haliday (hymenoptera: Braconidae) to combat *Pseudaletia (mythimna) separata* (Walk.) (Lepidoptera: Noctuidae). *New Zealand Journal of Agricultural Research*, 20: 255–258.
- Daane, K., Mills, N., Warner, K., Getz, C. & Welter, S. (2008) Interim report: Biological control of arthropod pests in california agriculture: Current status and future potential. *Biological control of arthropod pests in california agriculture*: 1-128.
- Daane, K.M., Sime, K.R., Fallon, J. & Cooper, M.L. (2007) Impacts of Argentine ants on mealybugs and their natural enemies in California's coastal vineyards. *Ecological Entomology*, 32: 583–596. DOI: 10.1111/j.1365-2311.2007.00910.x

- Dainese, M., Riedinger, V., Holzschuh, A., Kleijn, D. & Scheper, J. (2017) Managing trap-nesting bees as crop pollinators: spatiotemporal effects of floral resources and antagonists. *Journal of Applied Ecology*, 1–62.
- David, J. R., Gibert, P., Pla, E., Pétavy, G., Karan, D. & Moreteau, B. (1998) Cold stress tolerance in *Drosophila*: analysis of chill coma recovery in *D. melanogaster*. *Journal of Thermal Biology*, 5: 291–299.
- Day, R.L., Hickman, J.M., Sprague, R.I. & Wratten, S.D. (2015) Predatory hoverflies increase oviposition in response to colour stimuli offering no reward: Implications for biological control. *Basic and Applied Ecology*, 16: 544–552.
- DeBach, P. (1964) *Biological control of insect pests and weeds*. London: Chapman and Hall.
- Demoranville, C.J., Sandler, H.A. & Bicki, T. (2000) *Integrated Pest Management*. Amherst.
- Dent, D. (2000) *Insect Pest Management*. CABI Publishing, Cambridge.
- Donovan, B.J. (1989) Potential enemies of the introduced wasp parasitoid *Sphecohyphaga vesparum* (Hymenoptera : Ichneumonidae) in New Zealand. *New Zealand Journal of Zoology*, 16: 365–367.
- Eilenberg, J., Hajek, A. & Lomer, C. (2001) Suggestions for unifying the terminology in biological control. *BioControl*, 46: 387–400.
- De Lucia, E., Nabity, P., Zavala, J. & Berenbaum, M. (2012) Climate change: Resetting plant-insect interactions. *Plant Physiology*, 160: 1677–1685.
- De Sassi, C. de & Tylianakis, J.M. (2012) Climate change disproportionately increases herbivore over plant or parasitoid biomass. *PLoS ONE*, 7.
- De Schutter, O. (2009a) *Seed policies and the right to food: Enhancing agrobiodiversity, encouraging innovation*.
- De Schutter, O. (2009b) *The Role of the Right to Food in achieving Sustainable Global Food Security*. Rome.
- De Schutter, O.D. (2010) Report submitted by the special rapporteur on the right to food. United Nations General Assembly. Available at: <http://www2.ohchr.org/english/issues/food/docs/A-HRC-16-49.pdf> Date accessed: November 8 2017.

- Dhaliwal, G., Aroraa, R. & Dhawan, A. (2004) Crop losses due to insect pests in Indian agriculture: an update. *Indian Journal of Ecology*, 31(1): 1-7.
- Early, J.W. (1980) The Diapriidae (Hymenoptera) of the southern islands of New Zealand. *Journal of the Royal Society of New Zealand*, 10: 153–171.
- Early, J.W. & Horning, D.S.J. (1978) Two new wasp parasites (Hymenoptera : Diapriidae) of New Zealand Sciomyzidae (Diptera). *Journal of the Royal Society of New Zealand*, 8, 231–237.
- Faeth, S.H. (1985) Host leaf selection by leaf miners: interactions among three trophic levels. *Ecology*, 66: 870-875.
- Eschen, R., Britton, K., Brockerhoff, E., Burgess, T., Dalley, V., Epanchin-Niell, R.S., Gupta, K., Hardy, G., Huang, Y., Kenis, M., Kimani, E., Li, H.M., Olsen, S., Ormrod, R., Otieno, W., Sadof, C., Tadeu, E. & Theyse, M. (2015) International variation in phytosanitary legislation and regulations governing importation of plants for planting. *Environmental Science and Policy*, 51: 228–237.
- Estrada-Carmona, N., Hart, A.K., DeClerck, F.A.J., Harvey, C.A. & Milder, J.C. (2014) Integrated landscape management for agriculture, rural livelihoods, and ecosystem conservation: An assessment of experience from Latin America and the Caribbean. *Landscape and Urban Planning*, 129: 1–11.
- Fabinyi, M., Dressler, W.H. & Pido, M.D. (2016) Fish, trade and food security: Moving beyond “availability” discourse in marine conservation. *Human Ecology*: 1–12.
- Ferguson, R.S. & Lovell, S.T. (2014) Permaculture for agroecology: Design, movement, practice, and worldview. A review. *Agronomy for Sustainable Development*, 34: 251–274.
- Fiedler, A.K., Landis, D.A. & Wratten, S.D. (2008) Maximizing ecosystem services from conservation biological control: The role of habitat management. *Biological Control*, 45: 254–271.
- Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3: 294–299.
- Forbes, S.L., David, C., Ross, C., Wratten, S.D. & Fountain, J. (2009). Consumer attitudes regarding environmentally sustainable wine: an exploratory study of the New Zealand marketplace. *Journal of Cleaner Production*, 17(13):1195-1199. DOI 10.1016

Forrest, J.R. (2016) Complex responses of insect phenology to climate change. *Current Opinion in Insect Science*, 17: 49–54.

Foti, M. C., Rostas, M., Peri, E., Park, K. C., Slimani, T., Wratten, S. D. & Colazza, S. (2015) The role of plant volatiles in prediction of floral resource suitability: chemical ecology to enhance conservation biological control. In 4th International Entomophagous Insects Conference. 4 October 2015, Malaga, Spain.

Foti, M.C., Rostas, M., Peri, E., Park, K.C., Slimani, T., Wratten, S.D. & Colazza, S. (2017) Chemical ecology meets conservation biological control: identifying plant volatiles as predictors of floral resource suitability for an egg parasitoid of stink bugs. *Journal of Pest Science*, 90: 299–310.

Free, J.B. (1993) *Insect pollination of crops*, 2nd Edition. Academic Press, London, United Kingdom, 684 pp.

Fresia, P., Graneri, J. & Goni B. (2001) Anesthetic effects of two chemicals on the fertility of *Drosophila willistoni*. *Drosophila Informational Service*, 84: 141–142.

Gallien, L., Altermatt, F., Wiemers, M., Schweiger, O. & Zimmermann, N.E. (2016) Invasive plants threaten the least mobile butterflies in Switzerland. *Diversity and Distributions*, 185–195.

Gartaula, H., Patel, K., Johnson, D., Devkota, R., Khadka, K. & Chaudhary, P. (2016) From food security to food wellbeing: examining food security through the lens of food wellbeing in Nepal's rapidly changing agrarian landscape. *Agriculture and Human Values*: 1–17.

Gaud, W.S. (1968) *The green revolution: Accomplishments and apprehensions*. Speech given before the Society of International Development, Washington, D.C. USA. Available at:

<http://www.agbioworld.org/biotech-info/topics/borlaug/borlaug-green.html> Date accessed: November 1 2017.

Geertsema, W., Rossing, W.A., Landis, D.A., Bianchi, F.J., Rijn, P.C. Van, Schaminee, J.H., Tscharrntke, T. & van der Werf, W. (2016) Actionable knowledge for ecological intensification of agriculture. *Frontiers in Ecology and the Environment*, 14: 209–216.

Géneau, C.E., Wäckers, F.L., Luka, H., Daniel, C. & Balmer, O. (2012) Selective flowers to enhance biological control of cabbage pests by parasitoids. *Basic and Applied Ecology*, 13: 85–93. DOI: 10.1016/j.baae.2011.10.005

- Gillespie, M.A.K., Gurr, G.M. & Wratten, S.D. (2016) Beyond nectar provision: The other resource requirements of parasitoid biological control agents. *Entomologia Experimentalis et Applicata*, 159: 207–221.
- Gillespie, M., Wratten, S., Sedcole, R. & Colfer, R. (2011) Manipulating floral resources dispersion for hoverflies (Diptera: Syrphidae) in a California lettuce agro-ecosystem. *Biological Control*, 59: 215–220.
- Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., *et al.* (2010) Food security: the challenge of feeding 9 billion people. *Science*, 327(5967): 812–818. DOI: 10.1126/science.1185383
- Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M. & Toulmin, C. (2012) The Challenge of Food Security. *Science*, 327: 812.
- Goldson, S.L., McNeill, M.R., Phillips, C.B. & Proffitt, J.R. (1992) Host specificity testing and suitability of the parasitoid *Microctonus hyperodae* (Hym.: Braconidae, Euphorinae) as a biological control agent of *Listronotus bonariensis* (Col.: Curculionidae) in New Zealand. *Entomophaga*, 37: 483–498.
- Gonzalez-Chang, M., Wratten, S.D., Lefort, M. & Boyer, S. (2016) Food webs and biological control: A review of molecular tools used to reveal trophic interactions in agricultural systems. *Food Webs*, In Press.
- Gonzalvez, F.G. & Rodriguez-Girones, M.A. (2013) Seeing is believing: information content and behavioural response to visual and chemical cues. *Proceedings of the Royal Society of London: Biological Sciences*, 280: 20130886. DOI: 10.1098/rspb.2013.0886
- Gonthier, D.J. (2012) Do herbivores eavesdrop on ant chemical communication to avoid predation? *PLoS One*, 7: e28703. DOI: 10.1371/journal.pone.0028703
- Goulson, D. (2013) An overview of the environmental risks posed by neonicotinoid insecticides. *Journal of Applied Ecology*, 50: 977–987.
- Gregory PJ, Ingram JS, Campbell B, Goudriaan J, Hunt LA, Landsberg JJ, Linder S, Stafford Smith M, Sutherst RW, Valentin C. (1999) Managed production systems. In *The Terrestrial Biosphere and Global Change: Implications for Natural and Managed Ecosystems*, Walker B, Steffen W, Canadell J, & Ingram J. (eds). Cambridge University Press: New York; 229–270.
- Gurr, G., Wratten, S.D., Landis, D.A. & You, M. (2017) Habitat management to suppress pest populations: Progress and prospects. *Annual Review of Entomology*, 62: 91–109.

- Graves, R.A., Pearson, S.M. & Turner, M.G. (2017) Landscape dynamics of floral resources affect the supply of a biodiversity-dependent cultural ecosystem service. *Landscape Ecology*, 32: 415–428.
- Grehan, J.R. (1990) Invertebrate survey of some Island (matiu) and mokopuna Island, Wellington Harbour, New Zealand. *New Zealand Entomologist*, 13: 62–75.
- Grigoraki, L., Pipini, D., Labbe, P., Chaskopoulou, A., Weill, M. & Vontas, J. (2017) Carboxylesterase gene amplifications associated with insecticide resistance in *Aedes albopictus*: Geographical distribution and evolutionary origin. *PLOS Neglected Tropical Diseases*, 11: 1–13.
- Guedes, R.N.C. (2017) Insecticide resistance, control failure likelihood, & the first law of geography. *Pest Management Science*, 73: 479–484.
- Guo, F., Zhang, Z. & Zhao, Z. (1998) Pesticide resistance of *Tetranychus cinnabarinus* (Acari: Tetranychidae) in China: a review. *Systematic and Applied Acarology*, 3: 3–7.
- Gurr, G. M., D. M. Y. Read, J. L. A. Catindig, J. Cheng, J. Liu, L. P. Lan, & Heong, K.L. (2012) Parasitoids of the rice leafhopper *Cnaphalocrocis medinalis* and prospects for enhancing biological control with nectar plants. *Agricultural Forest Entomology*, 14: 1–12.
- Gurr, G., Wratten, S. D., & Altieri, M.A. (Eds.). (2004) *Ecological engineering for pest management: advances in habitat manipulation for arthropods*. CSIRO Publishing, Collingwood, Australia. 186 pp.
- Gurr, G., Wratten, S.D., Landis, D.A. & You, M. (2017) Habitat management to suppress pest populations: Progress and prospects. *Annual Review of Entomology*, 62: 91–109.
- Gurr, G.M., Lu, Z., Zheng, X., Xu, H., *et al.* (2016) Multi-country evidence that crop diversification promotes ecological intensification of agriculture. *Nature Plants*, 2: 16014.
DOI:10.1038/nplants.2016.14
- Gurr, G.M., Wratten, S.D. & Snyder, W.E., 2012. Biodiversity and insect pests. In: Gurr, G.M., Wratten, S.D., Snyder W., Read, D.M.Y. (Eds), *Biodiversity and Insect Pests: Key Issues for Sustainable Management*. Wiley-Blackwell, San Francisco, pp. 3-20
- Gurr, G.M. & You, M. (2016) Conservation biological control of pests in the molecular era: New opportunities to address old constraints. *Frontiers in plant science*, 6: 1255.
- Gurr, G.M., Zhu, Z.R. & You, M. (2015) *The big picture: Prospects for ecological engineering to guide the delivery of ecosystem services in global agriculture*. *Rice Planthoppers: Ecology, Management, Socio Economics and Policy*. Zhejiang University Press, Hangzhou.

- Gutierrez, A.P., Daane, K.M., Ponti, L., Walton, V.M. & Ellis, C.K. (2008) Prospective evaluation of the biological control of vine mealybug: Refuge effects and climate. *Journal of Applied Ecology*, 45: 524–536. DOI: 10.1111/j.1365-2664.2007.01356.x
- Grygorczyk, A., Turecek, J., & Lesschaeve, I. (2014) Consumer preferences for alternative pest management practices used during production of an edible and a non-edible greenhouse crop. *Journal of Pest Science*, 87: 249–258. DOI: 10.1007/s10340-013-0544-4
- Hackman, W. (1959) On the genus *Scaptomyza* Hardy (Dipt., Drosophilidae) with descriptions of new species from various parts of the World. *Acta Zoologica Fennica*, 97: 1–73.
- Haddad, N.M., Crutsinger, G.M., Gross, K., Haarstad, J. & Tilman, D. (2011) Plant diversity and the stability of foodwebs. *Ecology Letters*, 14: 42–46.
- Hagenbucher, S., Eisenring, M., Meissle, M. & Romeis, J. (2016) Interaction of transgenic and natural insect resistance mechanisms against *Spodoptera littoralis* in cotton. *Pest Management Science*.
- Halaj, J. & Wise, D.H. (2001) Terrestrial Trophic Cascades: How Much Do They Trickle? *The American Naturalist*, 157: 262–281.
- Hardy, C., Daly, N., Mobli, M., Morales, R. & King, G. (2013) Isolation of an orally active insecticidal toxin from the venom of an Australian tarantula. *PLOS One*, 8(9): e73136-e73136.
- Harris, R.J. (1991) Diet of the wasps *Vespula vulgaris* and *V. germanica* in honeydew beech forest of the south Island, New Zealand. *New Zealand Journal of Zoology*, 18: 159–169.
- Hatt, S., Lopes, T., Boeraeve, F., Chen, J. & Francis, F. (2016) Pest regulation and support of natural enemies in agriculture: Experimental evidence of within field wildflower strips. *Ecological Engineering*, 98: 240–245.
- Hegland, S.J., Nielsen, A., Lázaro, A., Bjerknes, A.L. & Totland, Ø. (2009) How does climate warming affect plant-pollinator interactions? *Ecology Letters*, 12: 184–195.
- Heinze, G. & Dunkler, D. (2008) Avoiding infinite estimates of time-dependent effects in small-sample survival studies. *Statistics in Medicine*, 27: 6455–6469.
- Heinze, G. & Ploner, M. (2002) SAS and SPLUS programs to perform Cox regression without convergence problems. *Computer Methods and Programs in Biomedicine*, 67: 217–223.

Heinze, G. & Ploner M. (2016) coxphf: Cox Regression with Firth's Penalized Likelihood. R package version 1.12.

Heinze, G. & Schemper M. (2001) A solution to the problem of monotone likelihood in Cox regression. *Biometrics*, 57: 114-119.

Hillebrandt, P.M. (1958) The Economic Theory of the Use of Pesticides. *Journal of Agricultural Economics*: 464–472.

Hemerik, L., Gebauer, K. & Meyhöfer, R. (2015) Comparison of the effect of predicted climate change on two agricultural pest-parasitoid systems. *Procedia Environmental Sciences*, 29: 75–76.

Hermann, S.L. & Landis, D.A. (2017) Scaling up our understanding of non-consumptive effects in insect systems. *Current Opinion in Insect Science*, 20: 54–60. DOI: 10.1016/j.cois.2017.03.010

Hermann, S. & Thaler, J. (2014) Prey perception of predation risk: volatile chemical cues mediate non-consumptive effects of a predator on a herbivorous insect. *Oecologia*, 176: 669–676. DOI: 10.1007/s00442-014-3069-5

Hoddle, M.S., Warner, K., Steggall, J. & Jetter, K.M. (2015) Classical biological control of invasive legacy crop pests: New technologies offer opportunities to revisit old pest problems in perennial tree crops. *Insects*, 6: 13–37.

Hoefler, C.D., Durso, L.C. & McIntyre, K.D. (2012) Chemical-mediated predator avoidance in the European house cricket (*Acheta domesticus*) is modulated by predator diet. *Ethology*, 118: 431–437. DOI: 10.1111/j.1439-0310.2012.02028.x

Hof, A.R. & Svahlin, A. (2015) The potential effect of climate change on the geographical distribution of insect pest species in the Swedish boreal forest. *Scandinavian Journal of Forest Research*, 31: 29–39.

Holland, J.M., Bianchi, F.J., Entling, M.H., Moonen, A.C., Smith, B.M. & Jeanneret, P. (2016) Structure, function and management of semi-natural habitats for conservation biological control: a review of European studies. *Pest Management Science*, 72(9): 1638–1651. DOI: 10.1002/ps.4318

Holzschuh, A., Dudenhöffer, J-H. & Tschardtke, T. (2012) Landscapes with wild bee habitats enhance pollination, fruit set and yield of sweet cherry. *Biological Conservation*, 153: 101–107. DOI: 10.1016/j.biocon.2012.04.032

Human Rights Council. (2017) Report of the Special Rapporteur on the right to food.

Ingram, J.S.I., Wright, H.L., Foster, L., Aldred, T., Barling, D., Benton, T.G., Berryman, P.M., Bestwick, C.S., Bows-Larkin, A., Brocklehurst, T.F., Buttriss, J., Casey J., Collins, H., Crossley, D.S., Dolan, C.S., Dowler, E., Edwards, R., Finney, K.J., Fitzpatrick J.L., Fowler, M., Garrett, D.A., Godfrey, J.E., Godley, A., Griffiths, W., Houlston, E.J., Kaiser, M.J., Kennard, R., Knox, J.W., Kuyk, A., Linter, B.R., Macdiarmid, J.I., Martindale, W., Mathers, J.C., McGonigle, D.F., Mead, A., Millar, S.J., Miller, A., Murray, C., Norton, I.T., Parry, S., Pollicino, M., Quedsted, T.E., Tassou, S., Terry, L.A., Tiffin, R., van de Graaf, P., Vorley, W., Westby, A. & Sutherland, W.J. (2013) Priority research questions for the UK food system. *Food Security*, 5: 617–636.

Irvin, N.A., Scarratt, S.L., Wratten, S.D., Frampton, C.M., Chapman, R.B. & Tylianakis, J.M. (2006) The effects of floral understoreys on parasitism of leafrollers (Lepidoptera: Tortricidae) on apples in New Zealand. *Agricultural and Forest Entomology*, 8: 25–34.

Jacobson, R.S., Matthews, R.W. & MacDonald, J.F. (1978) A systematic study of the *Vespula vulgaris* group with a description of a new yellowjacket species in eastern North America (Hymenoptera: Vespidae). *Annals of the Entomological Society of America*, 71: 299–312.

Iverson, A.L., Marín, L.E., Ennis, K.K., Gonthier, D.J., *et al.* (2014) Do polycultures promote win-wins or trade-offs in agricultural ecosystem services? A meta-analysis. *Journal of Applied Ecology*, 51(6): 1593–1602. DOI: 10.1111/1365-2664.12334

Jarvis, A., Lane, A. & Hijmans, R.J. (2008) The effect of climate change on crop wild relatives. *Agriculture, Ecosystems and Environment*, 126: 13–23.

Jax, K., Barton, D.N., Chan, K.M. a, Groot, R. De, Doyle, U., Eser, U., Gorg, C., Gomez-Baggethun, E., Griewald, Y., Haber, W., Haines-Young, R., Heink, U., Jahn, J., Joosten, H., Kerschbaumer, L., Korn, H., Luck, G.W., Matzdorf, B., Muraca, B., Nebhover, C., Norton, B., Ott, K., Potschin, M., Rauschmayer, F., von Haaren, & C., Wichmann, S.(2013) Ecosystem services and ethics. *Ecological Economics*, 93: 260–268.

Jervis, M.A., Kidd, N.A.C., Fitton, M.G., Huddleston, T. & Dawah, H.A. (1993) Flower-visiting by hymenopteran parasitoids. *Journal of Natural History*, 27: 67-105.

Johnsson, M., Wratten, S.D., Landis, D.A. & Gurr, G.M. (2010) Recent advances in conservation biological control of arthropods by arthropods. *Biological Control Special Issue*, 45(2): 172-175

- Jonsson, M., Buckley, H.L., Case, B.S., Wratten, S.D., Hale, R.J. & Didham, R.K. (2012) Agricultural intensification drives landscape-context effects on host–parasitoid interactions in agroecosystems. *Journal of Applied Ecology*, 49(3): 706–714. DOI: 10.1111/j.1365-2664.2012.02130.x
- Jetter, K., & Paine, T.D. (2004) Consumer preferences and willingness to pay for biological control in the urban landscape. *Biological Control*, 30: 312–322. DOI: 10.1016/j.biocontrol.2003.08.004
- Kaplan, I., McArt, S.H. & Thaler, J.S. (2014) Plant defenses and predation risk differentially shape patterns of consumption, growth, and digestive efficiency in a guild of leaf-chewing insects. *PLoS One*, 9: 20120948. DOI: 10.1371/journal.pone.0093714
- Karp, D.S., Rominger, A.J., Zook, J., Ranganathan, J., Ehrlich, P.R. & Daily, G.C. (2012) Intensive agriculture erodes β -diversity at large scales. *Ecology Letters*, 15: 963–970.
- Kauffmann, R. C., Y. Qian, A. Vogt, S. M. Sebt, A. D. Hamilton, & Carthew, R.W. (1995) Activated *Drosophila* Ras1 is selectively suppressed by isoprenyl transferase inhibitors. Proceedings of the National Academy of Sciences of the United States of America, 92: 10919–10923.
- Kean, J., Wratten, S., Tylianakis, J. & Barlow, N. (2008) The population consequences of natural enemy enhancement, and implications for conservation biological control. *Ecology Letters*, 6: 604–612.
- Khan, Z.R., Pickett, J.A., Berg, J.V.D., Wadhams, L.J. & Woodcock, C.M. (2000) Exploiting chemical ecology and species diversity: stem borer and striga control for maize and sorghum in Africa. *Pest Management Science*, 56(11): 957–962. DOI: 10.1002/1526-4998(200011)56:11<957::AID-PS236>3.0.CO;2-T
- Khan, Z., Midega, C., Pittchar, J., Pickett, J. & Bruce, T. (2011) Push–pull technology: a conservation agriculture approach for integrated management of insect pests, weeds and soil health in Africa. *International Journal of Agricultural Sustainability*, 9: 162–170.
- Kiptot, E. & Franzel, S. (2014) Voluntarism as an investment in human, social and financial capital: Evidence from a farmer-to-farmer extension program in Kenya. *Agriculture and Human Values*, 31: 231–243.
- Klick, J., Yang, W.Q. & Bruck, D.J. (2015) Marking *Drosophila suzukii* (Diptera: Drosophilidae) with Rubidium or ^{15}N . *Journal of Economic Entomology*, 108: 1447–1451.

- Kogan, M. (1998) Integrated pest management: historical perspectives and contemporary developments. *Annual Review of Entomology*, 43: 243–70.
- Konradsen, F., Hoek, W. van der, Cole, D.C., Hutchinson, G., Daisley, H., Singh, S. & Eddleston, M. (2003) Reducing acute poisoning in developing countries—options for restricting the availability of pesticides. *Toxicology*, 192: 249–261.
- Kremen, C. & Miles, A. (2012) Ecosystem Services in Biologically Diversified versus Conventional Farming Systems: Benefits, Externalities, and Trade-Offs. *Ecology and Society*, 17: 1–23.
- Landis, D.A., Wratten, S.D. & Gurr, G.M. (2000) Habitat management to conserve natural enemies of arthropod pests. *Annual review of entomology*, 175–201.
- Langer, V. (2001) The potential of leys and short rotation coppice hedges as reservoirs for parasitoids of cereal aphids in organic agriculture. *Agriculture, Ecosystems & Environment*, 87: 81–92. DOI: 10.1016/S0167-8809(00)00298-X
- Larson, B.M.H., Kevan, P.G. & Inouye, D.W. (2001) Flies and flowers: taxonomic diversity of anthophiles and pollinators. *The Canadian Entomologist*, 133: 439–465.
- Lavandero, B., Wratten, S., Shishehbor, P. & Worner, S. (2005) Enhancing the effectiveness of the parasitoid *Diadegma semiclausum* (Helen): Movement after use of nectar in the field. *Biological Control*, 34: 152–158.
- Lee, D.H., Nyrop, J.P. & Sanderson, J.P. (2014) Non-consumptive effects of the predatory beetle *Delphastus catalinae* (Coleoptera: Coccinellidae) on habitat use patterns of adult whitefly *Bemisia argentifolii* (Hemiptera: Aleyrodidae). *Applied Entomology and Zoology*, 49: 599–606. DOI: [10.1007/s13355-014-0294-7](https://doi.org/10.1007/s13355-014-0294-7)
- Lee, J.C. & Heimpel, G.E. (2008) Floral resources impact longevity and oviposition rate of a parasitoid in the field. *Journal of Animal Ecology*, 77: 565–572.
- Leibee, G.L. & Capinera, J.L. (1995) Pesticide resistance in Florida insects limits management options. *Florida Entomologist*, 78: 386–399.
- Lenteren, J.C. Van, Cock, M.J.W., Hoffmeister, T.S. & Sands, D.P.A. (2006) Host specificity in arthropod biological control, methods for testing and interpretation of the data. *Environmental Impact of Invertebrates for Biological Control of Arthropods: Methods and Risk Assessment*: 38–63.

- Letourneau, D.K., Armbrrecht, I., Rivera, B.S., Lerma, J.M., *et al.* (2011) Does plant diversity benefit agroecosystems? A synthetic review. *Ecological Applications*, 21: 9–21. DOI: 10.1890/09-2026.1
- Littlejohn, C.P., Curran, T.J., Hofmann, R.W. & Wratten, S.D. (2015) Farmland, food, and bioenergy crops need not compete for land. *Solutions*, 6(3): 36–50.
- Lu, Z.X., Zhu, P.Y., Gurr, G.M., Zheng, X.S., Read, D.M.Y., Heong, K.L., Yang, Y. & Xu, H. (2014) Mechanisms for flowering plants to benefit arthropod natural enemies of insect pests: Prospects for enhanced use in agriculture. *Insect Science*, 21: 1–12.
- Lynch, L., Hokkanen, H., Babendreier, D., Bigler, F., *et al.* (2001) Insect biological control and non-target effects: a European perspective. In: Wajnberg, E., Scott, J.K. & Quimby P.C. *Evaluating Indirect Ecological Effects of Biological Control*. CABI, New York, USA, 99–125.
- MacDonald, F.H., Walker, M.K. & Walker, G.P. (2011) Introducing *Asobara persimilis* for control of *Scaptomyza flava* in South Island brassica crops. *New Zealand Plant Protection*, 64: 282.
- Macfarlane, R.P. & Palma, R.L. (1987) The first record for *Melittobia australica* Girault in New Zealand and new host records for *Melittobia* (Eulophidae). *New Zealand of Zoology*, 14: 423–425.
- MacLeod, A., Wratten, S.D., Sotherton, N.W. & Thomas, M.B. (2004) ‘Beetle banks’ as refuges for beneficial arthropods in farmland: long-term changes in predator communities and habitat. *Agricultural Forest Entomology*, 6(2): 147–154. DOI: 10.1111/j.1461-9563.2004.00215.x
- MacMillan, H. A. & Sinclair, B.J. (2011) Mechanisms underlying insect chill-coma. *J. Insect Physiol.* 57: 12–20.
- Madeira, F. & Pons, X. (2015) Effects of weed density on the dispersal of *Orius majusculus* Reuter (Heteroptera: Anthracoridae) within maize. *Journal of Applied Entomology*, 139: 712–720.
- Madeira, F. & Pons, X. (2016) Rubidium marking reveals different patterns of movement in four ground beetle species (Col., Carabidae) between adjacent alfalfa and maize. *Agricultural and Forest Entomology*, 18: 99–107.
- Marohasy, J. (1998) The design and interpretation of host- specificity tests for weed biological control with particular reference to insect behaviour. *Biocontrol News and Information*, 19: 13–20.
- Mallet, J. (1989) The evolution of insecticide resistance: Have the insects won? *Tree*, 4: 336–340.

- Margulies, C., T. Tully, & Dubnau, J. (2005) Deconstructing memory in *Drosophila*. *Current Biology*, 15: 700–713.
- Marshall, K. E. & Sinclair B.J. (2012) The impacts of repeated cold exposure on insects. *Journal of Experimental Biology*, 215: 1607–1613.
- Martin, N.A. (2004) History of an invader, *Scaptomyza flava* (Fallen, 1823) (Diptera: Drosophilidae). *New Zealand Journal of Zoology*, 31: 27–32.
- Martin, N.A. (2012) Ecological studies of *Scaptomyza flava* (Fallen, 1823) (Diptera: Drosophilidae) and its parasitoids. *New Zealand Journal of Zoology*, 35: 58–67.
- Martinson, H.M. & Fagan, W.F. (2014) Trophic disruption: A meta-analysis of how habitat fragmentation affects resource consumption in terrestrial arthropod systems. *Ecology Letters*, 17: 1178–1189.
- Mauremooto, J.R., Wratten, S.D., Worner, S.P. & Fry, G.L.A. (1995) Permeability of hedgerows to predatory carabid beetles. *Agriculture, Ecosystems & Environment*, 52(2): 141–148. DOI: 10.1016/0167-8809(94)00548-S
- Maxwell, F.G. & Jennings, P.R. (1980) *Breeding plants resistant to insects*. John Wiley & Sons, New York, USA, 683 pp.
- McEvoy, P.B. (1996) Host specificity and biological pest control. *BioScience*, 46: 401–405.
- McLachlan, A.R.G. & Wratten, S.D. (2003) Abundance and species richness of field-margin and pasture spiders (Araneae) in Canterbury, New Zealand. *New Zealand Journal of Zoology*, 30: 57–67. DOI: 10.1080/03014223.2003.9518324
- Mensah, S., Veldtman, R. & Seifert, T. (2017) Potential supply of floral resources to managed honey bees in natural mistbelt forests. *Journal of Environmental Management*, 189: 160–167.
- Messelink, G.J., Bennison, J., Alomar, O., Ingegno, B.L., Tavella, L., Shipp, L., Palevsky, E. & Wackers F.L. (2014) Approaches to conserving natural enemy populations in greenhouse crops: Current methods and future prospects. *BioControl*, 59: 377–393.
- Morita, M., Ueda, T., Yoneda, T., Koyanagi, T. & Haga, T. (2007) Flonicamid, a novel insecticide with a rapid inhibitory effect on aphid feeding. *Pest Management Science*, 63: 969–973.

- Murage, A. W., Pittchar, J.O., Midega, C. A. O., Onyango, C.O. & Khan, Z.R. (2015) Gender specific perceptions and adoption of the climate-smart push–pull technology in eastern Africa. *Crop Protection*, 76: 83–91.
- Murdock, T.Q., Taylor, S.W., Flower, A., Mehlenbacher, A., Montenegro, A., Zwiers, F.W., Alfaro, A. & Spittlehouse, D.L. (2013) Pest outbreak distribution and forest management impacts in a changing climate in British Columbia. *Environmental Science and Policy*, 26: 75–89.
- Murray, T., Withers, T.M., Mansfield, S. & Bain, J. (2008) Distribution and current status of natural enemies of *Paropsis charybdis* in New Zealand. *New Zealand Plant Protection*, 61: 185–190.
- Myers, S.S., Smith, M.R., Guth, S., Golden, C.D., *et al.* (2017). Climate change and global food systems: Potential impacts on food security and undernutrition. *Annual Review of Public Health*, 38: 259–277. DOI: 10.1146/annurev-publhealth-031816-044356
- Nafziger, T. D. & Fadamiro, H.Y. (2011) Suitability of some farmscaping plants as nectar sources for the parasitoid wasp, *Microplitis croceipes* (Hymenoptera: Braconidae): effects on longevity and body nutrients. *Biological Control*, 56: 225–229.
- Nicolas, G., & Sillans, D. (1989) Immediate and latent effects of carbon dioxide on insects. *Annual Review of Entomology*, 34: 97–116.
- Nilson, T. L., B. J. Sinclair, & Roberts S.P. (2006) The effects of carbon dioxide anesthesia and anoxia on rapid cold-hardening and chill coma recovery in *Drosophila melanogaster*. *Journal of Insect Physiology*, 52: 1027–1033.
- Nilsson, U., Porcel, M., Swiergiel, W. & Wivstad, M. (2016) Habitat manipulation – as a pest management tool in vegetable and fruit cropping systems, with the focus on insects and mites. Uppsala.
- Noyes, J.S. (1988) *Fauna of New Zealand 13*. Science Information Publishing Centre, DSIR, Wellington.
- Ninkovic, V., Feng, Y., Olsson, U. & Pettersson, J. (2013) Ladybird footprints induce aphid avoidance behavior. *Biological Control*, 65: 63–71. DOI: 10.1016/j.biocontrol.2012.07.003
- Ohnishi, O. (1998) Search for the wild ancestor of buckwheat. I. Description of new *Fagopyrum* (Polygonaceae) species and their distribution in China and Himalayan hills. *Fagopyrum*, 15: 18–28.

- Oi, C. A., M. M. Lopez-Urbe, M. Cervini, & Del Lama, M.A. (2013) Non-lethal method of DNA sampling in euglossine bees supported by mark-recapture experiments and microsatellite genotyping. *Journal of Insect Conservation*, 17: 1071–1079.
- Olesen, J.E. & Bindi, M. (2002) Consequences of climate change for European agricultural productivity, land use and policy. *European Journal of Agronomy*, 16: 239–262.
- Opdam, P. & Wascher, D. (2004) Climate change meets habitat fragmentation: Linking landscape and biogeographical scale levels in research and conservation. *Biological Conservation*, 117: 285–297.
- Orre Gordon, G.U.S., Wratten, S.D., Jonsson, M., Simpson, M. & Hale, R. (2013) “Attract and reward”: Combining a herbivore-induced plant volatile with floral resource supplementation – Multi-trophic level effects. *Biological Control*, 64: 106–115.
- Pace, M., Cole, J., Carpenter, S. & Kitchell, J. (1999) Trophic cascades revealed in diverse ecosystems. *Trends in Ecology & Evolution*, 14: 483–488.
- Paredes, D., Cayuela, L., Gurr, G.M. & Campos, M. (2013) Effect of non-crop vegetation types on conservation biological control of pests in olive groves. *PeerJ*, 1: e116.
- Parker, J.D., Salminen, J.P. & Agrawal, A.A. (2010) Herbivory enhances positive effects of plant genotypic diversity. *Ecology Letters*, 13: 553–563.
- Parmesan, C., Ryrholm, N., Stefanescu, C., Hill, J.K., Thomas, C.D., Descimon, H., Huntley, B., Kaila, L., Kullberg, J., Tammaru, T., Tennett, W.J., Thomas, J.A. & Warren, M. (1999) Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Letters to Nature*, 399: 579–583.
- Pearse, A. (1980) *Seeds of plenty, seeds of want: social and economic implications of the green revolution*. In: Revisiting Sustainable Development. (Eds.) Utting, P. United Nations Institute for Social Development, Geneva, Switzerland, 139–157.
- Peng, S., Huang, J., Sheehy, J. E., Laza, R. C., Visperas, R. M., Zhong, X., Centeno, G.S., Khush, G.S. & Cassman, K. G. (2004). Rice yields decline with higher night temperature from global warming. *Proceedings of the National Academy of Sciences of the United States of America*, 101(27): 9971–9975.
- Perović, D.J., Gámez-Virués, S., Landis, D.A., Wäckers, F., Desneux, N., Wratten, S.D., You, M.-S. & Gurr, G.M. (2017). Managing biological control services through multi-trophic trait interactions:

review and guidelines for implementation at local and landscape scales. *Biological Reviews*. DOI: 10.1111/brv.12346

Perron, J. M., L. Huot, G. W. Corriveau, & Chawla S.S. (1972) Effects of carbon dioxide anaesthesia on *Drosophila melanogaster*. *Journal of Insect Physiology*, 18: 1869–1874.

Pimentel, D., Acquy, H., Biltonen, M., Rice, P., Silva, M., Nelson, J., Lipner, V., Giordano, S., Horowitz, A. & D'Amore, M. (1992) Environmental and Economic Costs of Pesticide Use. *BioScience*, 42: 750–760.

Poelman, E. H., Harvey, J. A., van Loon, J. J. A., Vet, L. E. M. & Dicke, M. (2013) Variation in herbivore-induced plant volatiles corresponds with spatial heterogeneity in the level of parasitoid competition and parasitoid exposure to hyperparasitism. *Functional Ecology*, 27: 1107–1116. doi:10.1111/1365-2435.12114

Porter, J., Arzberger, P.W., Braun, H., Bryant, P., Gage, S., Hansen, T., Hanson, P., Lin, C., Lin, F., Kratz, T., Michener, W., Shapiro, S. & Williams T. (2005) Wireless sensor networks for ecology. *Bioscience*, 55: 561–572.

Porter, J., Costanza, R., Sandhu, H., Sigsgaard, L. & Wratten, S. (2009). The Value of Producing Food, Energy, and Ecosystem Services within an Agro-Ecosystem. *AMBIO: A Journal of the Human Environment*, 38(4): 186–193. DOI: 10.1579/0044-7447-38.4.186

Price, B. W., C. S. Henry, A. C. Hall, A. Mochizuki, P. Duelli, & Brooks S.J. (2015) Singing from the grave: DNA from a 180 year old type specimen confirms the identity of *Chrysoperla carnea* (Stephens). *PLoS ONE*, 10: 2–9.

Prince, G.J. (1976) Laboratory biology of *Phaenocarpa persimilis* Papp (Braconidae: Alysiniinae), a parasitoid of *Drosophila*. *Australian Journal of Zoology*, 24: 249–264.

Pywell, R.F., Heard, M.S., Woodcock, B.A., Hinsley, S., *et al.* (2015) Wildlife-friendly farming increases crop yield: evidence for ecological intensification. *Proceedings of the Royal Society B: Biological Sciences*, 282: 20151740. DOI: 10.1098/rspb.2015.1740

Raman, A. & Withers, T.M. (2003) Oviposition by introduced *Ophelimus eucalypti* (Hymenoptera : Eulophidae) and morphogenesis of female-induced galls on *Eucalyptus saligna* (Myrtaceae) in New Zealand. *Bulletin of Entomological Research*, 93: 55–63.

- Ramsden, M.W., Menendez, R., Leather, S.R. & Wackers, F. (2014) Optimizing field margins for biocontrol services: The relative role of aphid abundance, annual floral resources, and overwinter habitat in enhancing aphid natural enemies. *Agriculture, Ecosystems and Environment*, 199: 94–104.
- Ratterman, D. M. (2003) Eliminating ether by using ice for *Drosophila* lab. Tested studies for laboratory teaching, pp. 259-265. In M. A. O'Donnell (ed.), Proceedings, 24th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 11–14 June 2003, Louisiana State University, Baton Rouge, LA.
- Reynolds, O. L. & Orchard, B.A. (2011) Effect of adult chill treatments on recovery, longevity and flight ability of Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: *Tephritidae*). *Bulletin of Entomological Research*, 101: 63–71.
- Ribbands, C. R. (1950) Changes in the behaviour of honey-bees following their recovery from anaesthesia. *Journal of Experimental Biology*, 27: 302–310.
- Richardson, J.S., Zhang, Y. & Marczak, L.B. (2010) Resource subsidies across the land-freshwater interface and responses in recipient communities. *River Research and Applications*, 26: 55–66.
- Ridgway, R.L., Tinney, J.C., MacGregor, J.T. & Starler, N.J. (1978) Pesticide use in agriculture. *Environmental Health Perspectives*, 27: 103–12.
- Roth, S.K. & Lindroth, R.L. (1995) Elevated atmospheric CO₂: Effects on phytochemistry, insect performance and insect-parasitoid interactions. *Global Change Biology*, 1: 173–182.
- Roubos, C.R. & Liburd, O.E. (2009) Effect of trap color on captures of grape root borer (Lepidoptera: Sesiidae) males and non-target insects 1. *Journal of Agricultural and Urban Entomology*, 25: 99–109.
- Roubos, C.R., Rodriguez-Saona, C. & Isaacs, R. (2014) Mitigating the effects of insecticides on arthropod biological control at field and landscape scales. *Biological Control*, 75: 28–38.
- Rusch, A., Birkhofer, K., Bommarco, R., Smith, H. G. & Ekbom, B. (2014) Management intensity at field and landscape levels affects the structure of generalist predator communities. *Oecologia*, 175(3): 971–83. DOI: 10.1007/s00442-014-2949-z
- Rusch, A., Chaplin-Kramer, R., Gardiner, M.M., Hawro, V., Holland, J., Landis, D., Thies, C., Tschardtke, T., Weisser, W.W., Winqvist, C., Woltz, M. & Bommarco, R. (2016) Agricultural landscape simplification reduces natural pest control: A quantitative synthesis. *Agriculture, Ecosystems and Environment*, 221: 198–204.

- Rusch, A., Valentin-Morison, M., Sarthou, J.-P. & Roger-Estrade, J. (2010) Biological control of insect pests in agroecosystems: effects of crop management, farming system, and seminatural habitats at the landscape scale: a review. *Advances in Agronomy*, Vol 109. 1st edn. Elsevier Inc.
- Rypstra, A.L. & Buddle, C.M. (2013) Spider silk reduces insect herbivory. *Biology Letters*, 9: 20120948. DOI: 10.1098/rsbl.2012.0948
- Sagarra, L.A., Vincent, C. & Stewart, R.K. (2001) Body size as an indicator of parasitoid quality in male and female *Anagyrus kamali* (Hymenoptera : Encyrtidae). *Bulletin of Entomological Research*, 91: 363–367.
- Sandhu, H.S., Crossman, N.D. & Smith, F.P. (2012) Ecosystem services and Australian agricultural enterprises. *Ecological Economics*, 74: 19–26.
- Sandhu, H., Porter, J. & Wratten, S. (2013) Experimental assessment of ecosystem services in agriculture. *Ecosystem services in agricultural and urban landscapes*: 122–135.
- Sandhu, H., Wratten, S., Costanza, R., Pretty, J., Porter, J.R. & Reganold, J. (2015) Significance and value of non-traded ecosystem services on farmland. *PeerJ*, 3: e762.
- Sandhu, H., Wratten, S., Porter, J.R., Costanza, R., Pretty, J. & Reganold, J.P. (2016) Mainstreaming ecosystem services into future farming. *Solutions*: 40–47.
- Scarratt, S.L., Wratten, S.D. & Shishehbor, P. (2008) Measuring parasitoid movement from floral resources in a vineyard. *Biological Control*, 46: 107–113.
- Schellhorn, N.A., Bianchi, F.J.J.A. & Hsu, C.L. (2014) Movement of entomophagous arthropods in agricultural landscapes: Links to pest suppression. *Annual Review of Entomology*, 59: 559–581.
- Scherber, C., Eisenhauer, N., Weisser, W.W., Schmid, B., Voigt, W., Fischer, M., Schulze, E., Roscher, C., Weigelt, A., Allan, E., Bebler, H., Bonkowski, M., Buchmann, N., Buscot, R., Clement, L.W., Ebeling, A., Engels, C., Halle, S., Kertscher, I., Klein, A., Koller, R., König, S., Kowalski, E., Kummer, V., Kuu, A., Lange, M., Lauterbach, D., Middlehoff, C., Migunova, V.D., Milcu, A., Müller, R., Partch, S., Petermann, J.S., Renker, C., Rottstock, T., Sabais, A., Scheu, S., Schumacher, J., Temperton, V.M. & Tschantke T. (2010) Bottom-up effects of plant diversity on multitrophic interactions in a biodiversity experiment. *Nature*, 468: 553–556.
- Schmitz, O.J. (1998) Direct and indirect effects of predation and predation risk in old-field interaction webs. *American Naturalist*, 151: 327–342. DOI: 10.1086/286122

- Schmitz, O.J. & Barton, B.T. (2014) Climate change effects on behavioral and physiological ecology of predator-prey interactions: Implications for conservation biological control. *Biological Control*, 75: 87–96.
- Schmitz, O.J., Hambäck, P. a. & Beckerman, A.P. (2000) Trophic Cascades in Terrestrial Systems: A Review of the Effects of Carnivore Removals on Plants. *The American Naturalist*, 155: 141–153.
- Sciencelab. 2013. Triethylamine MSDS. <https://www.sciencelab.com/msds.php?msdsId=9925315>.
- Seagraves, M.P., Kajita, Y., Weber, D.C., Obrycki, J.J. & Lundgren, J.G. (2011). Sugar feeding by coccinellids under field conditions: the effects of sugar sprays in soybean. *BioControl*, 56(3): 305–314. DOI: 10.1007/s10526-010-9337-3
- Semere, T. & Slater, F.M. (2007) Invertebrate populations in *Miscanthus* (*Miscanthus* × *giganteus*) and reed canary-grass (*Phalaris arundinacea*) fields. *Biomass and Bioenergy*, 31: 30–39. DOI: 10.1016/j.biombioe.2006.07.002
- Sendoya, S.F., Freitas, A.V.L. & Oliveira, P.S. (1996) Ants as selective agents on herbivore biology: effects on the behaviour of a non-myrmecophilous butterfly. *Journal of Animal Ecology*, 65: 205–210. DOI: 10.2307/5723.
- Sendoya, F., Freitas, V.L. & Oliveira, P.S. (2009) Egg-laying butterflies distinguish predaceous ants by sight. *American Naturalist*, 174: 134–140. DOI: 10.1086/599302
- Seraj, A.A. (1994) *Biology and host plant relationships of Scaptomyza flava leaf miner*. Ph.D. dissertation, Massey University, Palmerston North
- Shakeel, M., X. Z. He, N. A. Martin, A. Hanan, & Wang Q. (2009) Diurnal periodicity of adult eclosion, mating and oviposition of the European leafminer *Scaptomyza flava* (Fallén) (Diptera : Drosophilidae). *New Zealand Plant Protection*, 62: 80–85.
- Sharma, H. C. (2005). *Heliothis/Helicoverpa management: emerging trends and strategies for future research*: Oxford & IBH Publishing Co. Pvt. Ltd.
- Sharma, M., Ghosh, R. & Pande, S. (2015) Dry root rot (*Rhizoctonia bataticola* (Taub.) Butler): an emerging disease of chickpea – where do we stand? *Archives of Phytopathology and Plant Protection*, 48: 797–812.
- Shields, M.W., Tompkins, J.M., Saville, D.J., Meurk, C.D. & Wratten, S. (2016) Potential ecosystem

service delivery by endemic plants in New Zealand vineyards: Successes and prospects. *PeerJ*, 4: e2042. DOI: 10.7717/peerj.2042

Shields M.W., Buckley H.L & Wratten, S.D. (In press, 2018) Complementary approaches to evaluating the biological control refuge potential of a biofuel feedstock grass. *Agriculture, Ecosystem and Environment*.

Shields, M.W., Tompkins, J.-M., Saville, D.J., Meurk, C.D. & Wratten, S. (2016) Potential ecosystem service delivery by endemic plants in New Zealand vineyards: successes and prospects. *PeerJ*, 4: e2042.

Sidhu, S.C. & Wilson Rankin, E.E. (2016) Honey bees avoiding ant harassment at flowers using scent cues. *Environmental Entomology*, 45: 420-426. DOI: 10.1093/ee/nvv230

Sikulu-Lord, M. T., M. F. Maia, M. P. Milali, M. Henry, G. Mkandawile, E. A. Kho, R. A. Wirtz, L. E. Hugo, F. E. Dowell, & Devine. (2016) Rapid and non-destructive detection and identification of two strains of *Wolbachia* in *Aedes aegypti* by near-infrared spectroscopy. *PLOS Neglected Tropical Diseases*, 10: e0004759.

Simpson, M., Gurr, G.M., Simmons, A.T., Wratten, S.D., James, D.G., Leeson, G., Nicol, H.I. & Orre-Gordon, So. (2011) Attract and reward: Combining chemical ecology and habitat manipulation to enhance biological control in field crops. *Journal of Applied Ecology*, 48: 580–590.

Sivasubramaniam, W., Wratten, S.D. and Klimaszewski, J. (1997). Species composition, abundance, and activity of predatory arthropods in carrot fields, Canterbury, New Zealand. *New Zealand Journal of Zoology*, 24: 205-212.

Sivinski, J., Wahl, D., Holler, T., Dobai, S. Al & Sivinski, R. (2011) Conserving natural enemies with flowering plants: Estimating floral attractiveness to parasitic Hymenoptera and attraction's relationship to flower and plant morphology. *Biological Control*, 58: 208–214.

Smith, V. R., C. J. Vink, & Paterson. (2014) Carbon dioxide versus cold exposure for immobilising live redback spiders *Latrodectus hasseltii* Thorell, 1870 (Araneae: Theridiidae). *New Zealand Entomologist*, 38: 10–16.

Southwood, R. and Henderson, P.A. (2000) Ecological Methods. Blackwell Publishing Ltd, Victoria.

- Sprague, R.I., Boyer, S., Stevenson, G. & Wratten, S.D. (2016) Assessing pollinators' use of floral resource subsidies in agri-environment schemes: an illustration using *Phacelia tanacetifolia* and honeybees. *PeerJ*, 4: e2677, DOI: 10.7717/peerj.2677.
- Stevens, N.B., Stephens, C.J., Iqbal, M., Jennings, J.T., LaSalle, J. & Austin, A.D. (2007) What Wasp Is That? *Systematic Entomology*, 33: 210–211.
- Supply, C. B. (2012) Flynap safety data sheet. <http://www.carolina.com/teacher-resources/Document/msds-flynap/tr-msds-flynapghs.tr>.
- Syrett, P. (1990) The rose seed chalcid *Megastigmus aculeatus* Swederus (Hymenoptera: Torymidae) on sweet brier, *Rosa rubiginosa*, in the South Island tussock country. *New Zealand Entomologist*, 13: 34–38.
- Tena, A., Pekas, A., Cano, D., Wäckers, F.L. & Urbaneja, A. (2015) Sugar provisioning maximizes the biocontrol service of parasitoids. *Journal of Applied Ecology*, 52: 795–804. DOI: 10.1111/1365-2664.12426
- Thaler, J.S., Contreras, H. & Davidowitz, G. (2014) Effects of predation risk and plant resistance on *Manduca sexta* caterpillar feeding behaviour and physiology. *Ecological Entomology*, 39: 210–216. DOI: 10.1111/een.12086
- Therneau T. M. & Grambsch P.M. (2000) Modeling survival data: extending the Cox model. Springer, New York, NY. ISBN 0-387-98784-3.
- Thomas, M.B., Wratten, S.D. & Sotherton, N.W. (1991) Creation of island habitats in farmland to manipulate populations of beneficial arthropods: Predator densities and emigration. *Journal of Applied Ecology*, 3: 906–917.
- Thompson, R.M., Brose, U., Dunne, J.A., Hall, R.O., Hladyz, S., Kitching, R.L., Martinez, N.D., Rantala, H., Romanuk, T.N., Stouffer, D.B. & Tylianakis, J.M. (2012) Food webs: Reconciling the structure and function of biodiversity. *Trends in Ecology and Evolution*, 27: 689–697.
- Thomson, L.J., Macfadyen, S. & Hoffmann, A.A. (2010) Predicting the effects of climate change on natural enemies of agricultural pests. *Biological Control*, 52: 296–306.

- Tomasetto, F., Tylianakis, J.M., Reale, M., Wratten, S.D. and Goldson, S.L. (2016). Intensified agriculture favors evolved resistance to biological control. *Proceedings of the National Academy of Science*, DOI: 10.1073/pnas.1618416114.
- Tompkins, J.-M.L., Wratten, S.D. & Wäckers, F.L. (2010) Nectar to improve parasitoid fitness in biological control: does the sucrose:hexose ratio matter? *Basic and Applied Ecology*, 11: 264–271.
- Tilman, D. (1998) The greening of the green revolution. *Nature*, 396(6708): 211–212.
- Tilman, D., Balzer, C., Hill, J. & Befort, B.L. (2011). Global food demand and the sustainable intensification of agriculture. *Proceedings of the National Academy of Sciences*, 108(50): 20260–20264. DOI: 10.1073/pnas.1116437108
- Tittonell, P., Klerkx, L., Baudron, F., Felix, G.F., Ruggia, A., Apeldoorn, D. van, Dogliotti, S., Mapfumo, P. & Rossing, W.A.H. (2016) Ecological Intensification: local innovation to address global challenges. *Sustainable Agriculture Reviews*.
- Triplehorn, C.A. and Johnson, N.F. (2005) Study of Insects. Brooks/Cole, Belmont.
- Valentine, E.W. (1975) Additions and corrections to Hymenoptera hyperparasitic on aphids in New Zealand. *The New Zealand Entomologist*, 6: 59–61.
- Tscharntke, T., Bommarco, R., Clough, Y., Crist, T.O., Kleijn, D., Rand, T. A., Tylianakis, J.M., Nouhuys S. van, & Vidal, S. (2007) Conservation biological control and enemy diversity on a landscape scale. *Biological Control*, 43: 294–309.
- Tscharntke, T., Clough, Y., Wanger, T.C., Jackson, L., Motzke, I., Perfecto, I., Vandermeer, J. & Whitbread, A. (2012) Global food security, biodiversity conservation and the future of agricultural intensification. *Biological Conservation*, 151: 53–59.
- Tscharntke, T. & Hawkins, B.A. (2002) *Multitrophic level interactions*. Cambridge.
- Tscharntke, T., Karp, D.S., Chaplin-Kramer, R., Batary, P., DeClerck, F., Gratton, C., Hunt, L., Ives, A., Jonsson, M., Larsen, A., Martin, E.A., Martinez-Salinas, A., Meehan, T.D., O'Rourke, M., Poveda, Katja., Rosenheim, J.A., Rusch, A., Schellhorn, N., Wanger, T.C., Wratten, S. & Zhang, W. (2016) When natural habitat fails to enhance biological pest control - Five hypotheses. *Biological Conservation*.
- Tscharntke, T., Rand, T.A. & Bianchi, F.J. (2005) The landscape context of trophic interactions: insect spillover across the crop-noncrop interface. *Annales Zoologici Fennici*, 42: 421–432.

- Tschumi, M., Albrecht, M., Entling, M.H. & Jacot, K. (2015) High effectiveness of tailored flower strips in reducing pests and crop plant damage. *Proceedings of the Royal Society B: Biological Sciences*, 282: 20151369. DOI: 10.1098/rspb.2015.1369
- Turner, K.G., Anderson, S., Gonzalez, M., Costanza, R., Courville, S., Dominati, E., Kubiszewski, I., Ogilvy, S., Porfirio, L., Ratna, N., Sandhu, H., Sutton, P.C., Turner, G.M., Varennes, Y., Voinov, A., Wratten, S. (2014) Toward an integrated ecology and economics of land degradation and restoration : methods , data , and models. *Report to the ELD Project Data and Methodology Working Group*: 1–60.
- Tylianakis, J.M. & Binzer, A. (2014) Effects of global environmental changes on parasitoid-host food webs and biological control. *Biological Control*, 75: 77–86.
- Tylianakis, J.M., Didham, R.K. & Wratten, S.D. (2004) Improved fitness of aphid parasitoids receiving resource subsidies. *Ecology*, 85: 658–666.
- Tylianakis, J.M., Didham, R. K., Bascompte, J. & Wardle, D.A. (2008) Global change and species interactions in terrestrial ecosystems. *Ecology letters*, 11(12): 1351–1363. DOI: 10.1111/j.1461-0248.2008.01250.x
- Tylianakis, J.M. & Romo, C.M. (2010) Natural enemy diversity and biological control: Making sense of the context-dependency. *Basic and Applied Ecology*, 11: 657–668.
- Tylianakis, J.M., Tscharntke, T. & Lewis, O.T. (2007) Habitat modification alters the structure of tropical host-parasitoid food webs. *Nature*, 445: 202–205.
- Unruh, T.R., Pfannenstiel, R.S., Peters, C., Brunner, J.F. & Jones, V.P. (2012) Parasitism of leafrollers in Washington fruit orchards is enhanced by perimeter plantings of rose and strawberry. *Biological Control*, 62: 162–172.
- Van Dyck, H., Bonte, D., Puls, R., Gotthard, K. & Maes, D. (2015) The lost generation hypothesis: Could climate change drive ectotherms into a developmental trap? *Oikos*, 124: 54–61. DOI: 10.1111/oik.0206
- Villegas, C.M., Verdugo, J.A., Grez, A.A., Tapia, J. & Lavandero, B. (2013) Movement between crops and weeds: temporal refuges for aphidophagous insects in Central Chile. *Ciencia e investigación agraria*, 40: 317–326.

- Varennnes, Y.-D., Boyer, S. & Wratten, S.D. (2014) Un-nesting DNA Russian dolls – the potential for constructing food webs residual DNA in empty aphid mummies. *Molecular Ecology*, 23: 3925-3933. DOI: 10.1111/mec.12633
- Varennnes, Y.D., Boyer, S. & Wratten, S.D. (2016) Nectar from oilseed rape and floral subsidies enhances longevity of an aphid parasitoid more than does host honeydew. *BioControl*, 61: 631–638.
- Vattala, H., Wratten, S., Phillips, C. & Wackers, F. (2006) The influence of flower morphology and nectar quality on the longevity of a parasitoid biological control agent. *Biological Control*, 39: 179-185.
- Vogler, G. & Ocorr, K. (2009) Visualizing the beating heart in *Drosophila*. *Journal of Visualized Experiments*, 31: 6–8.
- Voinovich, N. D., N. P. Vaghina, & Reznik, S.Y. (2012) Effects of cold shock on host eggs parasitization by *Trichogramma buesi* Voegelé (Hymenoptera, Trichogrammatidae) females. *Entomologicheskoe Obozrenie*, 91: 681–690.
- Vong, J., Song, I. & Mandal, P. (2013) Application of ICT to improve rural livelihood in Vietnam. *International Journal of Electronic Finance*, 7(2): 132–145. DOI: 10.1007/978-981-287-347-7_14
- Wackers, F. (2004) Assessing the suitability of flowering herbs as parasitoid food sources: Flower attractiveness and nectar accessibility. *Biological Control*, 29: 307-314.
- Waddington, H., Snilstveit, B., Hombrados, J. G., Vojtkova, M., Anderson, J. & White, H. (2014) Farmer field schools for improving farming practices and farmer outcomes in low-and middle-income countries: a systematic review. *Campbell Systematic Reviews*, 10(6): 335 pp.
- War, A.R., Taggar, G.K., War, M.Y. & Hussain, B. (2016) Impact of climate change on insect pests, plant chemical ecology, tritrophic interactions and food production. *International Journal of Clinical and Biological Sciences*, 1: 16–29.
- Wardlow, L.R., Ludlamb, A.B. & Bradley, L.F. (1976) Pesticide Resistance in Glasshouse Whitefly. *Pesticide Science*, 7: 320–324.
- Warner, K.D. (2007) *Agroecology in action: Extending alternative agriculture through social networks*. The MIT Press, Cambridge.
- Wasserberg, G., White, L., Bullard, A., King, J. & Maxwell, R. (2013) Oviposition site selection in *Aedes albopictus* (Diptera: Culicidae): are the effects of predation risk and food level independent? *Journal*

of *Medical Entomology*, 50: 1159–1164. DOI: 10.1603/ME12275

Wedberg, S. E. & Clarke, N.A. (1947) A simple method for controlled experimentation on the passage of microorganisms through the digestive tract of insects. *Journal of Bacteriology*, 54: 447–450.

Weston, D.P., Holmes, R.W., You, J. & Lydy, M.J. (2005) Aquatic toxicity due to residential use of pyrethroid insecticides. *Environmental Science & Technology*, 39: 9778–84.

Weston, D.P., You, J. & Lydy, M.J. (2004) Distribution and toxicity of sediment-associated pesticides in agriculture-dominated water bodies of California's Central Valley. *Environmental Science & Technology*, 38: 2752–2759.

Wilkinson, V., Morris, T. & Hall, N. (2015) *The investor's guide to the New Zealand food & beverage industry*. Auckland.

Winkler, K., Wackers, F., Kaufman, L., Larraz, V. & van Lenteren, J. (2009) Nectar exploitation by herbivores and their parasitoids is a function of flower species and relative humidity. *Biological Control*, 50(3): 299–306.

Williams, C. & Kalmbach, E. (1943) Migration and fate of transported juvenile waterfowl. *The Journal of Wildlife Management*, 7(2): 163–169.

Willis, J. A. (1925) Effects of different tensions of carbon dioxide on certain Orthoptera. *The Biological Bulletin*, 48: 209–223.

Wilson, M.R. & Leather, S.R. (2012). The effect of past natural enemy activity on host-plant preference of two aphid species. *Entomologia Experimentalis et Applicata*, 144(2): 216–222. DOI: 10.1111/j.1570-7458.2012.01282.x

Wilson, C. & Tisdell, C. (2001) Why farmers continue to use pesticides despite environmental, health and sustainability costs. *Ecological Economics*, 39: 449–462.

Winding, A., Binnerup, S.J. & Pritchard, H. (2004) Non-target effects of bacterial biological control agents suppressing root pathogenic fungi. *FEMS Microbiology Ecology*, 47: 129–141. DOI: 10.1016/S0168-6496(03)00261-7

Winkler, K., Wäckers, F.L., Bukovinszky-Kiss, G. & Lenteren, J. van. (2006) Sugar resources are vital for *Diadegma semiclausum* fecundity under field conditions. *Basic and Applied Ecology*, 7: 133–140.

- Wood, G.R. & Saville, D.J. (2013) A geometric examination of linear model assumptions. *Australian and New Zealand Journal of Statistics*, 55: 285–303.
- Winkler, K., Wäckers, F.L., Termorshuizen, A.J. & van Lenteren, J.C. (2010) Assessing risks and benefits of floral supplements in conservation biological control. *BioControl*, 55(6): 719–727. DOI: 10.1007/s10526-010-9296-8
- Wolfenbarger, L.L., Naranjo, S.E., Lundgren, J.G., Bitzer, R.J. & Watrud, L.S. (2008) Bt crop effects on functional guilds of non-target arthropods: A meta-analysis. *PLoS ONE*, 3. DOI: 10.1371/journal.pone.0002118
- Wollaeger, H.E., Getter, K.L. & Behe, B.K. (2015) Consumer preferences for traditional, neonicotinoid-free, bee-friendly, or biological control pest management practices on floriculture crops. *HortScience* 50(5): 721–732.
- Woodward, A. & Porter, J.R. (2016). Food, hunger, health, and climate change. *The Lancet*, 387(10031): 1886–1887. DOI: 10.1016/S0140-6736(16)00349-4
- Worthen, W. B. & Moore J.L. (1991) Higher-order interactions and indirect effects: a resolution using laboratory *Drosophila* communities. *The American Naturalist*, 138: 1092–1104.
- Wratten, S.D. (1976) Searching by *Adalia bipunctata* (L.) and escape behaviour of its aphid and cicadellid prey on lime (*Tilia x vulgaris* Hayne). *Ecological Entomology*, 1: 139–142. DOI: 10.1111/j.1365-2311.1976.tb01215.x
- Wratten, S.D. Gonzalez-Chang, M., Shields, M.W., Nboyine, J., *et al.* (In press) Understanding the pathway from biodiversity to agro-ecological outcomes: A new, interactive approach. *Ecological Applications*.
- Wratten, S., Sanhu, H., Cullen, R. & Costanza, R. (2013) *Ecosystem Services in Agricultural and Urban Landscapes*. Wiley-Blackwell, Chichester.
- Wratten, S.D., Gillespie, M., Decourtye, A., Mader, E. & Desneux, N. (2012) Pollinator habitat enhancement: Benefits to other ecosystem services. *Agriculture, Ecosystems & Environment*, 159: 112–122.
- Wyckhuys, K.A.G., Bentley, J.W., Lie, R., Phuong, L.T.N. & Fredrix, M. (2017) Maximizing farm-level uptake and diffusion of biological control innovations in today's digital era. *BioControl*, 62: 1-16. DOI

- Yathom, S., Marcus, R., Chen, M., and Tal, S. (1988) Comparison of different positions and heights of yellow sticky traps for sampling populations of the leafminer *Liriomyza trifolii*. *Phytoparasitica* 16, 217–224.
- Zhang, J., K. E. Marshall, J. T. Westwood, M. S. Clark, & Sinclair, B.J. (2011) Divergent transcriptomic responses to repeated and single cold exposures in *Drosophila melanogaster*. *Journal of Experimental Biology*, 214: 4021–4029.
- Zhang, W., Ricketts, T.H., Kremen, C., Carney, K. & Swinton, S.M. (2007) Ecosystem services and dis-services to agriculture. *Ecological Economics*, 64(2): 253–260. DOI: 10.1016/j.ecolecon.2007.02.024
- Zhao, J., Guo, X., Tan, X., Desneux, N., Zappala, L., Zhang, F. & Wang, S. (2017) Using *Calendula officinalis* as a floral resource to enhance aphid and thrips suppression by the flower bug *Orius sauteri* (Hemiptera: Anthocoridae). *Pest Management Science*, 73: 515–520.
- Zehnder, G., Gurr, G.M., Kühne, S., Wade, M.R., Wratten, S.D. & Wyss, E. (2007) Arthropod pest management in organic crops. *Annual Review of Entomology*, 52: 57–80. DOI: 10.1146/annurev.ento.52.110405.091337
- Zhou, K., Huang, J., Deng, X., Werf, W. van der, Zhang, W., Lu, Y., Wu, K. & Wu, F. (2014) Effects of land use and insecticides on natural enemies of aphids in cotton: First evidence from smallholder agriculture in the North China Plain. *Agriculture, Ecosystems and Environment*, 183: 176–184.